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第60回 日本生理学会大会　昭和58年（1983）４月５日～７日　於大阪大学教養部校舎

第60回日本生理学会大会をかえりみて

第60回日本生理学会大会
当番幹事 中馬一郎・中山昭雄
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本大会は，第21回日本医学会総会と関連して，昭和58年4月5日(火)，6日(水)，7日(木)の3日間，豊中市待兼山の大阪大学教養部校舎および法学部・経済学部合同講義棟において開催された．また，本大会に先立ち，4月4日(月)に常任幹事会およびその他の諸委員会が，大阪市北区の関電会館で行われた．

大阪大学医学部が大会開催のお世話をするのは，昭和38年4月に第16回日本医学会総会が大阪で開催されたとき，久保秀雄教授が当番幹事を引き受けられて以来，20年ぶりのことである．今回の大会はまた還暦にあたる第60回でもあるので，何か新機軸をと考えたが結局次の二つに落着いた．一つはWiesel教授の特別講演で，もう一つは臨床医学との交流シンポジウムである．両方とも第21回日本医学会総会との関連で企画したものである．すなわち，Wiesel教授は日本医学会総会での特別講演のため招聘・来日されることがかなり早くからわかっていたので，この機会に生理学会大会においても特別講演をお願いすることにした．また，臨床医学との交流シンポジウムは，日本医学会総会出席のために多数の臨床医学者が来阪される機会を利用して，平素比較的疎になっている臨床系各学会との交流を盛んにし，臨床医学の各分野で現在注目されている諸問題について認識と理解を深め，生理学の研究の今後の発展に役立てたいと意図したものである．

演題数については，制限のなかった前回大会でも82%の研究単位が3題以下しか出題されていない実情をふまえ，3題に制限させていただくこととした．また，会場数は最低10は必要であろうとの予測から，交通の不便をしのんで本学豊中地区校舎を使用せざるを得ないこととなった．

以上の企画にもとづいて大会を開催したところ，参加者総数1,471名，発表演題数666（一般口演655，特別講演1，シンポジウム講演10)，会場数11という規模となった．各研究分野別の演題数（特別講演，シンポジウム講演を除く）は次のとおりである．研究方法10，分子生理16，細胞生理35，能動輸送18，興奮性膜48，シナプス・終板24，脊髄・末梢神経12，脳幹・間脳40，小脳8，終脳18，筋運動とその制御6，脳波・誘発電位14，行動表現22，神経化学11，視覚49，聴覚16，その他の感覚31，骨格筋25，平滑筋14，心筋29，自律神経系32，循環36，血液・腎・体液調節29，呼吸21，消化・吸収4，内分泌・生殖23，運動・体力・疲労12，環境・エネルギー代謝26，体温調節・発汗26，その他0，となった．これらの演題について，予稿集を編集し参加者に前もって頒布したことは従来どおりである．そのほかに，今回の大会での新しい試みとして，プログラムをハンディな小型冊子として編集作製した．これは大きな活字で印刷したこともあり，使い易いという点で好評であったと思っている．

大会運営については，今回はじめて2人座長制を採用してみた．その狙いは，どちらか1人が演壇に立つことが可能になることと，急用などで1人が来場できなくても進行には差支えない，ということであった．また，座長の1人に自動報時装置のスイッチを押してもらうことをお願いして，計時係を廃止することができた．その他運営については御満足いただけるよう意を尽したつもりであるが，駐車場が皆無であったこと，超満員となった会場があったことなど御不便をおかけした点が多々あったと思われる．御寛容を切にお願いする次第である．

大会会期中は幸いに天候に恵まれ，最終日には桜も満開となり，プログラム進行も大過なく経過した．とくに，Wiesel教授の特別講演と臨床医学との交流シンポジウムは多数の方々に御参加いただくことができ，主催者として心から喜んでいる．懇親会にも予期した以上の多数の方々が出席され，愉快なパーティとなった．

最後に，特別講演をお引受けいただいたWiesel教授，臨床医学との交流シンポジウムの講演者各位，一般口演の演者と司会者の方々，ならびに大会参加会員諸氏の御協力に対し深甚の謝意を表する次第である．

THE 60th ANNUAL MEETING
OF
THE PHYSIOLOGICAL SOCIETY OF JAPAN

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April 5 — 7, 1983

Osaka University Medical School

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1

A NEW DYNAMIC DIALYSIS METHOD TO DETERMINE THE BINDING CONSTANT OF A PROTEIN-LIGAND COMPLEX. UOZUMI, M., Div. of Environmental Health Research, Osaka Pref. Inst. of Public Health, Osaka 537, HASHIMOTO, M., HIGASHI, T. and ISOMOTO, A., Dept. of Physicochemical Physiology, Medical School, Osaka Univ., Osaka 530

A dialyzer, where the sink and the sample compartments were composed of narrow and long spaces, was constructed for the measurement of protein-ligand binding phenomena. Through the sink compartment, an elution buffer flowed while to exchange the containing ligand with the sample solution across the membrane, and the elution profile of the ligand concentration with respect to time was observed. Unlikely to the other dynamic dialysis methods, our method did not depend on the principle of mass-balance (Fick principle), because the ligand concentration in each compartment at a time was not unique, and the gradient of the concentration occurred in the longitudinal direction. In such system, partial differential equations of ligand concentration in time and position represented the ligand kinetics with the assumtion that the dissociation of the protein-ligand comlex occurred rapidly. According to the approximate solution of them, the elution profile of the ligand concentration in a single experiment could be easily analyzed without the strict knowledge of the membrane permeability and the reactor constants, to indicate the binding constant of the protein-ligand complex.

2

AN APPARATUS FOR RAPID CELLULOSE TUBING DIALYSIS. KAWAGUCHI, T., SHIMODE, M, and MATSUSHITA, H. Dept. of Physiology, Wakayama Medical College, Kyuban-Cho, Wakayama 640

An instrument is described for rapid dialysis using cellulose tubing. The principle of rapid dialysis is agitation of the inner solution of cellulose tubing, by an up and down movement of a fishing float. The movement of the float and the cellulose tubing is achieved by an external magnetic driving unit which permits use of the apparatus in sealed systems. The apparatus is constructed with 3 parts: a) small whell with 8 teeth contains a magnetic bar in the bottom, b) large wheel with 64 teeth to which cellulose bags containing samples and floats are fastened, c) holder. When magnetic stirrer set outside of the bucket which contained water and apparatus is started, the small toothed wheel start to rotate and then the large toothed wheel rotate. With every rotation of the large wheel, the float in the visking tube moved up and down one and agitate the inner solution of the bag.

3

A NEW IN VITRO MEASUREMENT OF ADHESIVENESS OF LEUKOCYTES USING A HEMATOCRIT GLASS CAPILLARY AND ITS EXPERIMENTAL APPLICATION. OHKUBO, C. and ASANO, M. Dept. of Physiological Hygiene, the Inst. of Public Health, Minato-ku, Tokyo 108

A simple and rapid in vitro method to study adhesiveness of leukocytes was developed. Heparinized whole blood of the rabbit was incubated in a hematocrit glass capillary at 37°C for 30 minutes under humid conditions. After incubation, the capillary was perfused with saline at a rate of 1.23ml/min for 60 seconds. Then the number of leukocytes adhering to a fixed length of the wall of the capillary was counted microscopically at x180 magnifications. The results were as follows: Mean of the maximum 3 counts of leukocytes was used as the representative for a capillary. Variation of the leukocyte counts between capillaries was relatively small. Two capillaries were used to measure a given blood specimen. No significant changes in the adhesiveness resulted from the storage within 5 hours.

Experimental application; two groups of 6 rabbits were fed a regular diet(control group) or that supplemented with 1.5% cholesterol(CH group) for 12 weeks and changes in the leukocyte adhesiveness were measured weekly in the manner mentioned above. The specimen of CH group showed a significant increase in the adhesiveness($p<0.005$) within one week of cholesterol feeding, although no effect was observed on the adhesiveness of leukocytes in control group.

4

CONSTRUCTION OF TWO-DIMENSIONAL LIPID BILAYER MEMBRANES FROM PREFORMED LIPID MONOLAYERS CONTAINING TRANSPORT CARRIERS. FUJITA,Y., OHSAWA,K. and HOSHI,T. Dept. of Physiol., Faculty of Med., Univ. of Tokyo, Bunkyo-ku, Tokyo 113

In an attempt to study transport kinetics of Na^+-linked cotransport in a two dimensional reconstituted system, procedures for constructing a planar lipid membrane from lipid (egg lecithin) monolayers and/or intestinal brush border membrane vesicles (BBMV) were evaluated. By measuring surface pressure, the limiting area of purified egg lecithin was first determined. The estimated value was 92 $Å^2$. Then, egg lecithin liposomes were prepared by the ethanol injection method in the presence of 1 M KCl and 2 mM $CaCl_2$. The majority of obtained liposomes had a relatively large diameter (200 - 300 nm) and unilamellar structure. The liposome suspension was slowly flowed along a glass rod onto water to be spreaded over the air-water interface. By this procedure, a suitable dense monolayer could be formed as indicated by expected increase in surface pressure. Similar procedures were applied to BBMV prepared from rabbit ileum by the $Ca^{++}(Mg^{++})$ precipitation method and further purified by the Sepharose-4B method of Carlson et al. (1983). Obtained BBMV were highly purified as indicated by high enrichment (x 30 - 50) and homogenous electronmicroscopic structure. Spreading the BBMV in the similar way caused an increase in surface pressure when the area of air-water interface was decreased by moving the mobile barrier.

5

THREE DIMENSIONAL MICRODRIVER OF THE RECORDING ELECTRODE FOR ANALYSIS OF NEURAL ACTIVITY IN THE CEREBRAL CORTEX. TAIRA, K., MATUSMOTO, N., SOMEI, K., SATO, T., and SUZUKI, T.A. Dept. of Oral Physiol., Sch. of Dent., Iwate Med. Univ., Morioka, Iwate 020

We have made a device for the three dimensional microelectrode drive to analyze more precisely the arrangement of cortical neurons and their dynamic behaviours. The device is composed of a cortical chamber and three hydraulic microdrivers corresponding to the three dimensional coordinates of x, y, and z. The chamber is covered with a plastic disk (16 mm in diameter) which is connected to the microdrivers of x and y axes, and which slides on a collar of the chamber (22 mm in diameter) maintaining liqid-tightness and minimizing the cortical pulsation. The electrode set on the microdriver z moves through the center of the disk. The movement of each hydraulic cylinder of the three microdrivers is controlled independently by a computer system and a tip of the electrode is directed to the desired positions in the cerebral cortex automatically or by keyboard manipulation. The maximum error between the desired and the controlled position of the electrode tip was 100 μm in the direction of the x and y axes, and 10 μm for the z axis. Utilizing the device to record neural activities in the somatosensory cortex of the cat, it was confirmed that recording of electrical activity of the single neuron could continue as long as the conventional techniques were used, and that more precise analysis of the functional columnar organization of the cerebral cortex could be achieved.

6

COMPUTER SIMULATION OF INPUT-OUTPUT RELATION IN MOTONEURON. NAKAJIMA, Y., HOMMA, S., MUSHA, T*. and OKAMOTO, Y*. Dept. Physiol., Sch. Med., Chiba Univ., Chiba 280 and *Dept. appl. Electr., Tokyo Ist. Technol., Yokohama 227

We simulated on a computer an impulse response excitatory postsynaptic potential (IR-EPSP) and augmentative EPSP which reflect a mono- and polysynaptic reflex activity in an alpha-motoneuron, respectively. Arrivals of Ia impulses at the presynaptic terminals were simulated as a random sequence of point events. Every time an Ia impulse arrives, the IR-EPSPs were linearly added on each other. As soon as the summed membrane potential attained the critical level for firing, the motoneuron model fired, of which process was an abrupt falling down of the membrane potential below to the resting membrane level. Then the potential was biased by a returning potential, which was assummed to rise linearly in time up to the augmentative EPSP level. This process was repeated and a sequence of motoneuronal spikes was obtained. An input (Ia impulses) and output (motoneuronal spikes) relation was formed by measuring time lags from the input to the output. It was found from this study that when a distribution of pre-existing membrane potential levels, from which an IR-EPSP with causation of spike generation starts to be added, is uniform, we can estimate the rising phase and time-to-peak of the IR-EPSP, and that when the augmentative EPSP level is enough high to elicit spikes by itself, the pre-exisitng membrane potential distribution becomes uniform.

7

UNIDIMENSIONAL LATENCY-TOPOGRAPH AND ITS APPLICATION. HOMMA, S., NAKAJIMA, Y., IWATA, K. and MIZOTE, M. Dept. Physiol., Sch. Med., Chiba Univ., Chiba 280

The ventral and dorsal root of the rat was placed on 12 electrodes arranged side by side at 0.4 mm intervals. Impulse conduction along the fiber was displayed using unidimensional latency-topograph, which corresponds to the relation between latency and electrode distance. The relation revealed step-like displacement, which implies saltatory conduction of the impulse. Since the distance between plateaux corresponded to internodal length, the conduction velocity could be calculated from the length and the time differences between the plateaux. It was found from 36 observations that i) mean internodal length was 0.92 ± 0.13 mm, ii) mean conduction velocity was 76.1 ± 20.7 m/s, and iii) the correlation coefficient of conduction velocity and internodal length was statistically significant at $r=0.38$ ($P<0.025$), so the longer the internodal length, the higher the conduction velocity. This analysis was applied to rats with neuropathy, caused by administration of Clioquinol. The internodal length and conduction velocity were compared with those of normal. It was found that demyelination due to Clioquinol occured, and that shortening of myelin length due to the demyelination caused decrease of conduction velocity in the Clioquinol treated rats.

8

THE EFFECT OF THE ELECTRODE SPACING ON THE VARIANCE OF INTEGRATED EMG. YOSHIDA, M., AKAZAWA, K. and FUJII, K.* Dept. of Electrical Engineering, Fac. of Engineering, OSAKA University, Suita, OSAKA 565

The method of decreasing the variance of the integrated EMG (IEMG) is studied in consideration of the bipolar electrode spacing. The EMG is picked up from the human biceps bracnii of 7 heathly subjects. The subject keeps his isometric force at constant level (10% of MVC) for 20 s. IEMG is calculated with 500 ms integration time. The variance of IEMG is got from 40 data. The results from 25 cases of 7 subjects show that the narrower electrode spacing makes the smaller variance. The variance of IEMG picked up by 5 mm electrode spacing is 49% of that in case of 35 mm electrode spacing. This relation is analyzed by using a model of surface EMG generation. This model is based on the motor units activities. The results of analysis show that the short dulation of the surface motor unit action potentials make the small variance of IEMG. Thus, the narrow electrode spacing makes the accuracy of IEMG good.

9

EVALUATION OF THE DIELECTRIC DISPERSION APPROACH BY USE OF A SINGLE SPHERICAL BILAYER MEMBRANE. ASAMI,K. and IRIMAJIRI,A. Dept. of Physiol. Kochi Med. Sch., Nankoku, Kochi

Single spherical bilayer membranes of the Pagano-Thompson type(Pagano,R. and Thompson, T.E. (1967) Biochim. Biophys. Acta 144, 666-669), formed from monooleyl phosphate and cholesterol dissolved in $CHCl_3/CH_3OH$/n-decane, were subjected to a fast impedance analysis of high precision. Dielectric behavior of the whole system, as monitored from outside the spherical membrane, was sensitive to changes in the membrane state from the thick colored to the thin black state. With a spherical membrane 2 - 3 mm in diameter formed in the sample cavity containing 0.12 ml of 10 mM NaCl, the former state was characterized by a dielectric dispersion having dielectric increment ($\Delta\varepsilon$) of some 10^2 and characteristic frequency (f_c) around 10^6 Hz, while the latter by $\Delta\varepsilon \simeq 10^5$ and $f_c \simeq 10^3$ Hz. Complex plane plots for both dispersions traced semicircles, indicating that the present system may be unequivocally analyzed to yield spherical radius and membrane capacity (C_M) on the basis of a well-established dielectric theory. C_M for the thin membranes has thus been determined to be 0.54 $\mu F \cdot cm^{-2}$ in excellent agreement with a separate determination on planar membranes. The applicability of the present type of spherical membranes under dielectric monitoring to the study of membrane fusion or of exocytosis is suggested.

10

EXPERIMENTAL STUDY IN RELIEVING PAIN WITH THE METHOD OF VIBRATION BY GRINDING. KUMABE,M.,SUZUKI,S.,KUMABE,J*,YUYAMA,N. and SEKI,S. Dept. of Oral Physiology, Kanagawa Dental College,Inaoka 82, Yokosuka and * Dept. of Precision Engineering, Utsunomiya Univ.,Utsunomiya

Pain is a sensation received by the brain when abnormal symptons develop through the peripheral nervous system, or the impulse-conducting system. Then the sensation, touch off by the memories of past experiences or pains, will generate such reactions of pain as a sudden change of expression,fright,perspiration,tachy-cardia and metabolism abnormality. Pain is also defese mechanism, a self-protective physiologicl reaction, which is followed by various reactions in order to defend against irritation. The kinetic treatment is currently used to grind the teeth by a grinding instrument. Therefore, it is important to reduce the forces of grinding, which would result in less movement on the teeth and avoid the impact of grinding to affect the nervous systems. The theory of vibration by grinding is considered for the purpose of alleviating pain and discomfort without using medication. We are pleased to report that the results of our clinical experiments based on the theory have been satisfactory.

11

PROPERTIES OF HEMOGLOBIN BOUND TO RED CELL MEMBRANE.

IMAI, K., TSUNESHIGE, A.* and TYUMA, I. Dept. of Physicochemical Physiology, Osaka University Medical School, Nakanoshima, Osaka 530

Hemoglobin (Hb) is known to bind to red cell membrane. The binding is reversible and electrostatic in nature. We investigated properties of membrane-bound Hb and compared them with properties of free Hb. After hemolysis and succeeding repeated washing of the membrane with dilute phosphate buffer (pH 6.5), a small amount of Hb remained bound to the membrane. The Hb component liberated from the membrane upon further washing showed the same isoelectric focusing pattern and oxygen binding properties as those of the bulk Hb. The visible-range absorption spectra of deoxy, oxy, aquomet, and cyanmet forms were also indistinguishable between the two Hb components. Red cell membrane completely freed of Hb was prepared by washing with dilute phosphate buffers of pH 8. The Hb recombined with the membrane showed a lowered oxygen affinity, reduced cooperativity, Bohr effect, and effect of DPG or IHP compared to those of membrane-free Hb. The lowered oxygen affinity of the membrane-bound Hb is inconsistent with the fact that the affinity of oxyHb for membrane is higher than that of deoxyHb.

12

STRUCTURAL AND FUNCTIONAL CHARACTERIZATIONS OF CHICKEN GIZZARD MYOGLOBIN.

ENOKI, Y., KAWASE, M., SAKATA, S. and TOMITA, S. 2nd Dept. of Physiology, Nara Medical University, Kashihara, Nara 634

Presence of myoglobin (Mb) has been known in smooth muscles of chicken gizzard, but the structure and function has never been studied. The present study was intended to clarify the properties of this protein and to determine whether it is identical to Mb in the skelet muscles or not. The Mb was prepared from the gizzard extract by gel filtration combined with chromatofocusing and was homogeneous as judged from the gel electrophoresis. Its isoelectric point was estimated as 7.82±0.05. Spectroscopic properties of the oxy-, carboxy-, deoxy- and met-derivaties in ultraviolet to visible regions were very similar to those of mammalian Mb's. The molecular weight estimated by equilibrium sedimentation was 1.8×10^4, which coincided with that by the iron content. The protein exhibited a hyperbolic oxygen binding curve with no such allosteric properties as the Bohr and anion effect. The binding was exothermic and the $\Delta H°$ was -16.6 Kcal/mole. As for all these characteristics, the gizzard and skelet muscle Mb's were little different. Their amino acid compositions and peptide maps were also completely identical.

13

FLUORESCENCE LIFE TIME WITH A DIMERIZATION OF HEMATOPORPHYRIN DERIVATIVE.

AIZAWA, K., SAKAI, S., O'HATA, S., KATO, H.[1], HAYATA, Y.[1], YAMASHITA, M.[2], and SATO, T.[2] Dept. of Physiology and [1]Surgery Tokyo Medical college Shinjuku-ku Tokyo 160. [2]Laser Research Section Electrotechnical Laboratory Umezono Niharigun Ibaraki 305.

Hematoporphyrin derivative (HpD) have been two absorption bands [B band (solet band) and Q band] in the ultraviolet-visible region for π-π^* transition. The solet band is found to be much more sensitive to change from monomer to dimer than the visible region. We measured the 2nd order derivative spectra at solet band (monomer type at 396nm, dimer type at 365nm) for different concentrations of HpD. At the below a 4×10^{-7}mol/l of HpD, the molar extinction coefficient of the monomer have been higer than dimer and those value was reversed above the same concentration. The Q band have been separated 4th peacks at 505nm (Qy 0-0),535nm (Qy 0-1),565nm (Qx 0-0),and 610nm (Qx 0-0) for a 3000cm^{-1}. At the below a 4×10^{-7}mol/l , the Qy 0-0 have not a lage intensity and also Qy 0-1 have a small intensity but the Qy 0-0 and Qy 0-1 have been formed a large intensity each other above the same concentration. Fluorescence quenching of HpD in the PBS solution occurs in the picosecond time region with a increasing concentration using a streak camera operating in synchronus mode locked CW dye laser. It's quenching originated from non radiative transition which the low level of the excited state of B region changed the lower energy level than the excited the Q region with a dimer formation of the HpD.

14

SPECTROFLUOROMETRIC DETERMINATION OF PORPHYRIN RETENTION IN TISSUES. KAKIUCHI, Y., ARAI, T. and KOYAMA, T. Div. of Physiol., Res. Inst. Appl. Electr., Hokkaido Univ., Kita-ku, Sapporo 060

A selective affinity of hematoporphyrin derivatives (HPD with three different radicals) to neoplastic tissues was examined by using a pulsed laser spectrofluorometer (reported previously). To follow the time course of HPD accumulation in tissues hamsters with adenocarcinom and rats with brain tumor were sacrificed at time intervals of 3 to 96 hours after intravenous injection of HPD (20 mg/Kg), then tumors and such organs as liver, kidney and muscle were immediately removed to examine. Samples presented similar pattern of spectra, which distributed over the range from 400 to 800 nm forming two peaks at 470 nm and at around 620 nm. The former peak was used as a measure reflecting amount of intracellular NADH and the latter the HPD retained in tissues respectively. Maximal retention of HPD appeared in 12 hours after the administration and the tumors retained HPD longer than 96 hours, whereas normal organs cleared quickly. Any HPD was not found in normal brain because of blockage by its blood-brain barrier, however dramatic uptake of HPD was observed in brain tumor. Difference in radicals of HPD brought a wide variety of both retentivity and selectivity, and their phototherapeutic ability to cancer was discussed.

15

STUDIES ON RESOLUTION OF HUMAN MERCAPTALBUMIN AND NONMERCAPTALBUMIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND LOW-ANGLE LASER LIGHT SCATTERING. SOGAMI, M., NAGAOKA, S., ERA, S. and KUWATA, K. Dept. of physiology, School of Medicine, Gifu University, Gifu 500

(1) Gel-exclusion high-performance liquid chromatographic (HPLC) analysis of bovine plasma albumin (BPA) and human serum albumin (HSA) were carried out using PGP 2000 column (0.10 M Na phosphate buffer 0.30 M NaCl, pH 6.86, 25°). Because of slight difference between BPA-resin interaction and that of HSA, elution volume of HSA was larger than that of BPA, resulting in the clear resolution of HSA from BPA and the resolution of HSA into 2 components. (2) Low-angle laser light scattering technique in the combination with HPLC analysis on PGP 2000 column indicated 2 componenets of HSA to be monomer. (3) Chemical modification of SH group of HSA indicated that the principal component is mercaptalbumin and secondary one mainly nonmercaptalbumin.

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ANALYSIS OF FLUORESCENCE AND CD OF THE COMPLEX OF 1-ANILINO-8-NAPHTHALENE SULFONATE WITH BOVINE PLASMA ALBUMIN IN ACID-INDUCED ISOMERIZATION. NOZAKI, M*, ERA, S., NAGAOKA, S. AND SOGAMI, M. Dept. of Pharmacology* and Dept. of Physiology, School of Medicine, Gifu University, Gifu 500

The acid-induced isomerization of bovine plasma albumin (BPA) was studied by measuring fluorescence, fluorescence polarization and induced CD of 1-anilino-8-naphthalene sulfonate (ANS) bound with BPA. Most of changes in ANS fluorescence increase and reciprocal of fluorescence polarization at 460 nm decrease correlated with the N-F_1 transition. Rotational relaxation times of the N- and F_1-forms in 0.05 M $NaClO_4$ were 107 and 126 nsec, respectively, indicating changes in shape or hydration of BPA as results of conformational changes in the N-F_1 transition. Rotational strengths at 280 nm of the N-, F_1-, F_2- and E-forms in 0.05 M $NaClO_4$ were - 0.293, - 0.201, - 0.148 and - 0.163 DBM, respectively. It suggested that bound ANS of BPA-ANS complex might undergo less asymmetric environment in the N-F transition.

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STUDIES ON THE ACID-INDUCED TRANSITION OF BOVINE MERCAPTALBUMIN BY 360 MHz ^{1}H-NMR.
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Bovine mercaptalbumin (BMA) showed the two-step transition in the acidic region, one corresponding to the N-F transition (0.10 M NaCl, pH 4.5 ~ 3.7) and the other to the acid-expansion. The rigidity and hydration of the N- and F-forms in deuterated solution were studied using 360 MHz ^{1}H-NMR. (1) the rigidity of BMA was estimated by the spin-diffusion technique (saturation transfer). The cross-relaxation times (T_{IS}) obtained by irradiation at f_2 (ppm) were as follows :

	T_{IS} (sec), f_2 = - 7.18 ppm				T_{IS} (sec), f_2 = 2.40 ppm		
	CH_3	LYS	AROM	HIS	CH_3	LYS	HIS
N-form (pD 5.32)	0.43	0.55	0.44	0.75	0.32	0.39	0.57
F-form (pD 4.03)	0.53	0.68	0.83	3.85	0.46	0.53	0.88

The obtained T_{IS} values clearly indicate that the rigidity of the N-form is greater than that of the F-form. (2) Amounts of hydration of the N- and F-forms were estimated by measuring the longitudinal relaxation time (T_1) of HDO at various BMA concentrations. The amount of hydrated water (g/g) of the N- and F-forms were 1.32 and 0.99 g/g respectively.

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CONFORMATION OF ANGIOTENSIN II. UYEDA, M., IWAI, T., FUJIWARA, T. AND TANIMOTO, O., Dept. of Physiology, School of Medicine, Gifu University, Tsukasa-machi, Gifu and Dept. of Applied Physics, Faculty of Engineering, Osaka City University, Osaka

The conformation properties of angiotensin II studied by means of conformation energy calculation, by circular dichroism (CD) spectra calculation and by n.m.r. parameters calculation. The computed CD had shown to agree very well to experimental results but n.m.r. vicinal coupling constants of some conformations were not. The peptide CD of angiotensin II are known to have random and β form in water and in more hydrohobic solvent, respectively. Although computed CD spectra of several allowed comformationhad shown to be that of β formed peptide, the computed conformation had none of β form or β turn. Random form CD similar to experimental CD in water was obtained from the computation based on generation of random conformation which restricted both dihedral angle ϕ and ψ to between $-\pi$ and 0. The result suggests that in water solution angiotensin II have weight averaged conformation of some of allowed comformation. The result that the comformation of four c-terminal amino residue sequence, all of which consist of hydrohobic amino acid, had similar conformation between allowed lowest energy conformations suggests this part of the peptide bind to receptor protein.

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^{31}P-NMR STUDIES ON THE ORDERED STRUCTURE OF INTACT SPINAL MYELIN. NISHIKAWA, H. and YOSHIZAKI, K. Dept. of Physiology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602

The phosphorus chemical shift anisotropy of ^{31}P-NMR was applied to the intact myelin in order to prove its ordered structure, and the motions of its phospholipids were analized under the various conditions. The cow spinal white matter was placed in the magnetic field in the direction that the fiber axes were parallel or perpendicular to it. ^{31}P-NMR spectra were recorded by Jeol PFT 100 Spectrometer at 40.29 MHz.

^{31}P-NMR spectrum in parallel contained a broad resoance line at +11.1 ppm, but one in perpendicular showed flat shape at the position. The spectral change indicates that the white matter is constituted orderly with phospholipids like artificial lipid bilayers. The nuclear Overhauser effect(NOE) was 1.3. The value is nearly equal to the ones of artificial membranes. T_1 and T_2 relaxation times of phospholipids in the white matter were 383 msec and 2.9 msec in normal Krebs-Ringer's solution respectively, and their T_1 and T_2 relaxation times were 695 msec and 2.5 msec in the solution containing 10 mM EDTA respectively. The difference in T_1 suggests that rotation of phospholipids is affected by divalent cations. T_2 was not changed by EDTA, and not by various pH also. The transition temperature was 50°C - 60°C that is due to the spectral changes.

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STUDY ON FLUIDITY OF ERYTHROCYTE MEMBRANES BY NANOSECOND LASER FLUOROMETER. ARAISO, T. KOYAMA, T., ARAI, T. AND SHINDO, Y. Division of Physiology, Research Institute of Applied Electricity, Hokkaido University, Sappro, 060.

Fluidity and structural compactness of the erythrocyte membranes were investigated by nanosecond fluorescence techniques. Motions of a fluorescent hydrocarbon probe DPH which is trapped in the lipid bilayer of the membranes offer such rheological and structural information (Kinoshita, K. et al. (1977) Biophys. J.). The DPH molecules in the membranes make wobbling motions in sterically restricted area with characteristec diffusion rate relation microviscosity of the lipid layers. The wobbling angle (θ) varing with the size of the restricted area is considered as a compactness parameter. Using fluorescence depolarization method, these motions can be detected as decrease of fluorescence anisotropy of DPH. For normal erythrocytes, the values of those parameters are determined as follows : $\theta = 30°$, diffusion rate = $5 \times 10^7 s^{-1}$ and microviscosity = 0.8 poise. Those results indicate that the human erythrocytes have compact structure but still keep high fluidity. The measurements were performed with newly designed nanosecond fluorometer composed of pulsed N_2-laser, optics, photomultipliers, time-to-amplitude convertor with 0.5 nsec time resolution and a microcomputer for measurement controlling and data processing.

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SELECTIVE SPIN LABELING OF MALEIMIDE REAGENT TO RAT LIVER MITOCHONDRIA. KUMAZAWA, N., TSUJIMOTO, T., KANASHIRO, M*, MIURA, R*, MIYAKE, Y*, Dept. of Physiol., Wakayama Med. Coll., Wakayama 640, Dept. of Biochem*, National Cardiovascular Center, Suita, Osaka 565

N-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolidinyl)maleimide (MSL) was incorporated into rat liver mitochondria and nitroxide radical was found to decay considerably. The incorporation was blocked by N-ethylmaleimide (NEM) but not by p-chloromercuribenzoic acid (PCMB). Spin labeled fatty acid derivatives, 2-(3-carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinyloxyl and 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl were also incorporated and the nitroxide radical decayed. However, their incorporation was not blocked by NEM or PCMB. The ESR spectrum of 3-carboxyl-2,2,5,5-tetramethyl-pyrroline-1-oxyl did not change on reaction with mitochondria. The results suggest that MSL is labeled selectively in the mitochondrial membrane through those SH groups that are not reactive to PCMB, and the labeled nitroxide radical is reduced in sutu. The incorporation of MSL was dependent on the concentration of MSL used. ADP acceleration of mitochondrial oxygen uptake with succinate was inhibited by labelig the mitochondria with MSL without loss of the electron transferring activity.

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A STUDY OF CHLORPROMAZINE AS A FLAVIN ANTAGONIST. Shiga,k., Nishina,Y., and Watari,H. Dept. of Molecular Physiology, National Institute For Physiological Sciences

The interaction between chlorpromazine (CPZ) and riboflavin binding protein (RBP) from egg white was studied by the spectrophotometric and spectrofluorometric methods.

CPZ combined with RBP in a 1:1 molar ratio and the dissociation constant was determined to be 3.8×10^{-5} M at pH 7, 25° C. Flavins such as riboflavin (Rf) and lumiflavin were released from the complexes with RBP by the addition of CPZ.

The mechanism of flavin-releasing was mainly due to the competitive binding between CPZ and a isoalloxazine ring of flavins to RBP.

Some biological phenomena induced by CPZ were explained based on this mechanism.

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A RESONANCE RAMAN STUDY ON THE FLAVOENZYME D-AMINO ACID OXIDASE CATALYTIC INTERMEDIATES. NISHINA, Y., SHIGA, K., WATARI, H., MIURA, R.,[1]* OHTA, M.,[1]* MIYAKE, Y.,[1]* TOJO, H.,[2] AND YAMANO, T.[2] National Institute for Physiological Sciences, Okazaki, [1]Dept. of Biochem., Res. Inst., National Cardiovascular Center, Suita, Osaka, [2]Dept. of Biochem., Osaka Univ. Medical School, Osaka.

Resonance Raman (RR) spectra were measured for the catalytic intermediates (purple intermediates) of D-amino acid oxidase reconstituted with isotopically labelled FAD's, i.e., [4a-^{13}C]-, [4,10a-$^{13}C_2$]-, [2-^{13}C]-, [5-^{15}N]-, and [1,3-$^{15}N_2$]-FAD. The RR bands around 1605 cm^{-1} with D-alanine or D-proline as a substrate undergo isotopic shifts upon [4a-^{13}C]- and [4, 10a-$^{13}C_2$]-labelling. These bands are assigned to vibrational modes associated with C(10a)=C(4a)-C(4)=O moiety of reduced flavin. RR spectra were also obtained using isotopically labelled substrates, i.e., [1-^{13}C]-, [2-^{13}C]-, [3-^{13}C]-, [^{15}N]-, and [3,3,3-d_3]- alanine; [1-^{13}C]- and [^{15}N]-proline; [1-^{13}C]- and [^{15}N]lysine. The large isotopic shifts of the 1692 cm^{-1} band upon [2-^{13}C]- and [^{15}N]-labelled alanines confirm that the mode at 1692 cm^{-1} has a major component of C=N stratching of an imino acid, α-imino propionic acid. The isotopic shifts of the 1363 cm^{-1} band upon [1-^{13}C]- and [3-^{13}C]-labelled alanines, and the disappearance of the band upon [3,3,3-d_3]-labelled alanine indicate that the mode at 1363 cm^{-1} is assigned as the CO_2^- symmetric stretch + CH_3 symmetric deformation.

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NOTE ON THE EXACT SOLUTION OF MEMBRANE POTENTIAL EQUATION. SHINAGAWA, Y.**, SHINAGAWA, YASUKO and SOGAMI, I.* Dept. of Physiology, Kyoto University Medical School, Sakyo-ku, Kyoto 606 and Dept. of Physics, Kyoto Sangyo University, Kita-ku, Kyoto 603* (Present address: Dept. of Physiology, Nippon Medical School, Bunkyo-ku, Tokyo 113**)

An exact solution of the one-dimensional Poisson equation of the membrane and the dipole layer is obtained by means of the rational function method (Hirota's method). The exact solution revealed the explorsion of solution at the membrane surface, which corresponds to the explorsion of the exact solution of the Nernst-Planck-Poisson equations for ion transport across membrane [1)2)].

1) Y. Shinagawa: J. theor. Biol. 81, 333 (1979)
2) Y. Shinagawa: J. theor. Biol. 83, 359 (1980)

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INHIBITION OF NERVE GROWTH FACTOR(NGF)-INDUCED RNA SYNTHESIS BY 5-CHLORO-7-IODO-8-HYDROXYQUINOLINE (CHINOFORM). HORI, S., KAYANUMA, K. and OHTANI, S. Dept.Neurochem., Tokyo Metropolitan Institute for Neurosciences, Fuchu-city, Tokyo 183

Administration of chinoform, in vivo, for a long period develops a systematic neuronal degeneration(SMON, subacute myelo-optico-neuropathy). Previously we reported that chinoform specifically inhibit RNA synthesis induced by NGF in neonatal rat superior cervical ganglion, and suggested that chinoform affect DNA, RNA and protein syntheses by different mechanism from its metal chelating property. In this paper, we studied the toxic mechanism of chinoform on DNA, RNA and protein syntheses. Chinoform did not affect the uptake velocity into ganglion of thymidine, uridine and leucine, which are precuosors of DNA, RNA and protein, respectively, and did not stimulate the degradation of synthesized RNA, in vitro. These results suggest that chinoform inhibit RNA synthesis itself and interact with NGF action on the process of RNA synthesis in ganglion.

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ON THE RNA CODON

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In order to clear the significance of RNA codon of amino acid, we paid attention to the special case of UUC→ Phe under the consideration of the matrix $S=\begin{pmatrix} U & U \\ C & * \end{pmatrix}$. S is the matrix of the linear transformation of coordinate axis,that is X'=SX. The molecular weigt of U is 112 and that of C is 111 respectively. The characteristic equation, the characteristic value λ and the characteristic vector e{l,m } can be estimated respectively, when the value of * is given according to the formula of Se=λe. If the value of * is 1514, the matrix is expressed as following, $S=\begin{pmatrix} 112 & 112 \\ 111 & 1514 \end{pmatrix}$. From this formula, we get λ=103. 1881 as the suitable proper value. Further, we get the vlues of the components of the characteristic vector as follews, cosθ= l=112G and sinθ= m= -8.8119G. Where G is the constant.

The rotational angle of the coordinate axis is estimated as -4.4986° This value is strictly coincidence with $[\alpha]_D^{25}$ (5N:HCl)=-4.5°of Phe.

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POTENTIAL CHANGES IN SEA URCHIN EGGS INSEMINATED WITH OYSTER SPERM.
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It has been found that many sperm of the oyster(*Crassostrea gigas*) enter a sea urchin egg (*Temnopleurus handwicki*), and yet the egg often does not elevate the fertilization membrane; that is, the egg is not activated (fertilized). Utilizing this phenomenon we tried to record a potential change associated with sperm entry, separating from the "activation potential" (AVP) associated with egg activation. These nonactivated eggs showed sporadic, transient depolarizations (TDs), each of which consisted of an action potential and the following slow depolarization up to +5 mV. The total duration was about 30 sec. The TD is considered as a "sperm entry potential" because the frequency of TDs was dependent on the sperm concentration and the number of recorded TDs was approximately consistent with the number of sperm nuclei found in serial sections of the same egg. After several TDs had occurred, the AVP was induced by additional insemination with much higher density of sperm or by 5 uM Ca ionophore. It is suggested that heterologous sperm is less effective than homologous sperm in inducing intracellular Ca release which results in egg activation.

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POTENTIAL CHANGES IN HAMSTER EGGS INSEMINATED WITH MOUSE SPERM.
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Zona-free hamster egg was inseminated with mouse sperm and potential change was recorded. When inseminated with mouse sperm in the concentration of 5-10 x 10^4 cells/ml, the hamster egg exhibited periodic hyperpolarization. Compared with homologous sperm, the initiation of periodic hyperpolarization was delayed and the frequency was low. Inseminated with diluted (less than 1/5) sperm suspension, the hamster egg showed no periodic hyperpolarization in spite of the penetration of mouse sperm. Histological examination revealed that the second meiotic division was resumed in the eggs showing periodic hyperpolarization, whereas it was not resumed in the eggs showing no periodic hyperpolarization. It was concluded that the periodic hyperpolarization in the hamster egg during fertilization was closely related to the resumption of the second meiotic division. The delay in the initiation as well as the longer intervals of periodic hyperpolarization may indicate that the heterologous mouse sperm cannot increase Ca influx as much as the homologous hamster sperm can.

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REGULATION OF INTRACELLULAR MONOVALENT CATIONS IN HELA CELLS.
IKEHARA, T., YAMAGUCHI, H., HOSOKAWA, K., MIYAMOTO, H. and *SAKAI, T., Dept. of Physiology, School of Medicine, The University of Tokushima, Tokushima 770 and *Dept. of Physiology, Kinki University School of Medicine, Sayama, Osaka 589.

The purpose of the present study is to control intracellular monovalent cations by ionophores. Monensin and valinomycin have little effect on the cation levels. However, nystatin, when applied for one hour at 0 °C; strongly increased permeation of the cations through the plasma membrane of HeLa cells. The titration curve shows that the reagent becomes effective at concentrations above 5 μg/ml. The rate coefficient of cation fluxes reveals that nystatin (50 μg/ml) enhances effluxes more significantly than influxes and Na^+ fluxes than K^+ fluxes as well. The intracellular Na^+ levels seem to be slightly changed within 30 min after removal of nystatin but there is no change any more from 30 min on. When we chill cells in the ice-cold media containing various concentrations of Na^+ (40-1200 mM) and K^+ keeping the concentration ratio at 2 to 1, intracellular Na^+ levels are changed logarithmically but K^+ can be maintained at a high constant level.

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SPERM-ACTIVATING PEPTIDES FROM ECHINODERM EGGS——ITS MODE OF ACTION. OHTAKE, H., KOGA, M. AND IKADAI, H.* Dept. Physiol., Dokkyo Univ. Sch. Med., Mibu, Tochigi; *Dept. Develop. Biol. National Institute for Basic Biology, Okazaki, Aichi

On the process of fertilization in echinoids, the first event is the penetration of spermatozoa into the jelly coat of the eggs. The pH value within the jelly coat seems to be lower than that of normal sea water (pH 8.2) by approx. 1-2 pH units, because the jelly coat contains high amounts of sialoglycoprotein and fucose sulfate-rich glycoprotein. The respiration and motility of echinoids spermatozoa are strongly reduced at such pH values as this and the rate of their movement in the jelly coat should, therefore, be markedly lower than that attained in sea water. Recently, sperm-activating peptides, which stimulate the respiration and motility at lower pH values than that of sea water, were purified from the jelly coat of sea-urchin eggs. The activation of spermatozoa can also be caused with an ionophore monensin which catalyzes a Na^+-H^+ exchange across plasma membrane. These findings suggest that the peptides catalyze the exchange of Na^+ of high concentration outside the cell for H^+ of high concentration inside the cell. Therefore, by the aid of this peptides, the spermatozoa should be able to maintain their high activities attained in sea water during their passage through the slightly acidic environment of the jelly coat to reach the egg surface. Similar peptides are also obtained from starfish eggs.

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POLARITY OF HEPATOCYTE AND THE DISTRIBUTION OF PLASMA MEMBRANE PROTEINS. TAKEMURA, S.*, MATSUURA, S., OMORI, K., OMORI, K.*, SAMESHIMA, Y.* and TASHIRO, Y. Dept. of Physiology and Internal Medicine, Kansai Medical.University, Moriguchi, Osaka 570

Distribution of 5'-nucleotidase and (Na^+, K^+)-ATPase on hepatocyte cell surface was investigated by using direct ferritin immunoelectron microscopy. Prefixed hepatocytes were isolated from rat liver homogenate after perfusion fixation with 0.6 % glutaraldehyde and incubated with ferritin antibody conjugates. It was revealed that 5'-nucleotidase is mainly localized on the microvilli of bile canalicular surface in large clusters and on those of sinusoidal surface in small clusters. The particle density on the former was 4-5 times higher than that on the latter. (Na^+, K^+)-ATPase also showed similar heterogeneous distribution excepting that this enzyme was destributed rather homogeneously on both the bile canalicular and sinusoidal surface.

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EFFECT OF BOMBESIN AND NEUROPEPTIDE Y STIMULATING RESPIRATORY CHAIN IN BULLFROG BRAIN. SAITO, T., *SAITO, A. and KANNO, T. Dept. of Physiol., Fac. Vet. Med., Hokkaido Univ. Kita-ku, Sapporo 060 and *Dept. of Vet. Physiol., Obihiro Univ. Agr.& Vet.Med.,Obihiro 080

Bullfrog (body weight 350-450 g) brains were perfused with HEPES Ringer solution via cannula inserted into internal carotid arteries with the aid of a roller pump at a constant flow of 2 ml/min at a room temperature. Redox state and oxygen consumption in the cerebral hemisphere was measured with a scanning organ spectrophotometer, a fluorometer and a Clark-style oxygen electrode. Vascular infusion of Bombesin and Neuropeptide Y (NPY) (both lnM) after preceeding perfusion of 300 mM *l*-arabinose for 30 sec increased NAD(P)H fluorescence and reduced cytochrome $a(a_3)$, b and $c + c_1$. These peptides increased oxygen consumption in the isolated brain. A solution of 300 mM *l*-arabinose *per se* had little, if any, effect on the redox state of respiratory chain and oxygen consumption. These data indicate that Bombesin and NPY act on the respiratory chain in the bullfrog brain under condition that the blood-brain barrier was probably opened by the preceeding perfusion with a solution containing *l*-arabinose . These changes of respiratory chain may correlated with activities of neuron or neuroglia.

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INFLUENCES OF pH VALUE OF PERFUSING SOLUTION ON OXIDATIVE PHOSPHORYLATION PROCESS AND SECRETORY PROCESS IN THE ISOLATED PERFUSED RAT PANCREAS. ASADA, N. and KANNO, T. Dept. Physiol., Fac. Vet. Med., Hokkaido Univ., Kita-ku, Sapporo 060

Influences of extracellular pH value (pHe) on the oxidative phosphorylation process and secretory process in the pancreatic acinar cell were examined. Lowering the pHe value induced simultaneous oxidation of cytochrome $a(a_3)$, b and $c + c_1$; and decrease in oxygen consumption. On the contrary, raising the pHe value reduced cytochromes, and slightly increased oxygen consumption. Lowering pHe value to 6.8 or 6.0 increased the ATP content, inhibited secretory responses(pancreatic juice flow and enzyme output) induced by 0.1 nM CCK-8. Raising the pH value always inhibited the secretory responses. Lowering the pHe value to 6.0 reduced the Ca^{2+}-dependent secretory responses. The present study suggests that lowering the pHe value acts directly on the respiratory chain to amplify the proton gradient between the cytosol and intramitochondrial space, and inhibits Ca^{2+}-activated exocytosis of the zymogen granules.

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CALCIUM EFFLUX FROM THE BOVINE CHROMAFFIN CELLS MAINTAINED IN PRIMARY CULTURE. SORIMACHI, M. AND NISHIMURA, S. Dept. of Physiology, Kagoshima University Medical School, Kagoshima 890.

The efflux rate of ^{45}Ca previously taken up by the chromaffin cells has been studied. Ca efflux during continuous exposure to a Ca-free, Mg(1 mM)-containing medium decreased by approx. 30 % when external NaCl was replaced by choline Cl, Tris Cl or sucrose, but its extent of reduction was smallest(mean 12 %) in Li-substituted medium. Ca reintroduction under this condition increased the rate of Ca efflux. The Ca-activated Ca efflux was described by a simple Michaelis-Menten function with the dissociation constant of 50 μM. The extent of increased efflux by Ca was comparable to that in the absence of Na, showing that Ca-activated Ca efflux is stimulated by Na removal. Ca-activated Ca efflux was also greatly increased by pretreatment with a medium lacking Mg or containing ouabain, a condition under which internal Na was shown to increase markedly. Na removal at the time of Ca reintroduction further increased Ca efflux during exposure to a medium lacking Mg.

These results suggest that Ca efflux is subdivided into three components, one which is activated by external Na, another by external Ca and a third which is independent of Na and Ca.

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EXTERNAL K STIMULATES ADRENO-MEDULLARY CATECHOLAMINE SECRETION INDUCED BY INTERNAL Na-DEPENDENT Ca INFLUX MECHANISM. SORIMACHI, M. AND NISHIMURA, S. Dept. of Physiology, Kagoshima University Medical School, Kagoshima 890.

Cat adrenal glands were isolated and retrogradely perfused with a modified Locke's medium containing 2 mM Ca. Exposure of the adrenal to a medium containing ouabain (50μM) caused a time-dependent increase of secretion. During this period, raising external K from 5 mM to 10 mM caused an abrupt increase of secretion rate, which immediately diminished following reducing the concentration of K to 5 mM. When Na was partially replaced by Tris or choline(in the presence of nicotinic antagonist), both the rate of increased secretion by ouabain and that induced by 10 mM K became smaller. A small increase of K also increased the rate of secretion observed during exposure to a medium containing cyanide or monoiodoacetate, which is previously shown to reduce indirectly Na pumping activity. Furthermore, the responses to Ca reintroduction during exposure to a medium containing 70 mM or 100 mM Na were potentiated in the presence of 10 mM K.

These result thus suggest that a small increment of K stimulates Ca influx in exchange for internal Na, which is set in motion by a rise in internal Na.

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SECRETORY STIMULATION ON ^{45}Ca AND ^{86}Rb EFFLUXES AND AMYLASE RELEASE FROM THE SALIVARY SEGMENTS OF RAT. KATOH, K. AND *NISHIYAMA, A. Dept. of Anim. Physiol., Facul. of Agri., and *First Dept. of Physiol., Sch. of Med., Tohoku Univ., Sendai 980

In order to investigate the secretory mechanisms in salivary gland acinar cells, ^{45}Ca and ^{86}Rb effluxes and amylase release induced by secretory stimulation were measured in the superfused segments of rat parotid or submaxillary gland. In the parotid gland, ACh (5.5×10^{-6} M) or phenylephrine (5×10^{-5} M) increased amylase release accompanied with an increase of ^{45}Ca and ^{86}Rb effluxes. On the other hand, isoprenaline (2.5×10^{-6} M) markedly increased amylase release, which was not accompanied with increase in ^{45}Ca or ^{86}Rb efflux. However, isoprenaline significantly increased ^{86}Rb efflux in the presence of ouabain (10^{-4} M). In submaxillary gland, dibutyryl cyclic AMP (3 mM) or salbutamol (3.5×10^{-4} M) did not increase ^{86}Rb efflux even in the presence of ouabain. The results of ACh and phenylephrine are compatible with the general hypothesis that these agonists increase the intracellular Ca^{2+} concentration resulting in increase of K permeability and amylase release. The results of isoprenaline indicate that the mechanism of its action seems more complex than our previous hypothesis that isoprenaline directly activates Na-K pump and amylase release through elevating the intracellular cyclic AMP concentration.

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EFFECT OF ELECTRICAL FIELD STIMULATION ON K NET FLUX IN SUPERFUSED RAT SUBMAXILLARY GLAND. KANEKO, K., KATOH, K., SUZUKI, Y. and *NISHIYAMA, A. Dept. of Physiology, Yamagata University School of Medicine, Yamagata 990-23, *Dept. of Physiology, Tohoku University School of Medicine, Sendai 980.

Effects of neural excitation on net K flux from superfused segments of rat submaxillary gland were investigated by electrical field stimulation (FS). FS (80 V, 2 msec, 16 Hz, 1 min) evoked a biphasic response in K net flux consisting of initial K release ($1\ \mu mole \cdot g^{-1} \cdot min^{-1}$) followed by K uptake ($0.5\ \mu mole \cdot g^{-1} \cdot min^{-1}$). Both the K release and K uptake evoked by the FS were completely blocked by TTX (10^{-7} g/ml), but they were little affected by hexamethonium (3.7×10^{-4} M). Addition of atropine (1.4×10^{-6} M) blocked the FS-evoked K release, but did not block the K uptake. The FS-evoked K uptake appeared in the presence of atropine was blocked by propranolol (5×10^{-6} M) or ouabain (10^{-4} M). This K uptake was not observed in the gland pretreated with 6-hydroxydopamine (100 mg/kg). From our present and previous (Nishiyama, 1980) findings, we conclude that the FS excites both of post-ganglionic cholinergic and adrenergic fibers and that the cholinergic transmitter (ACh) increases the acinar cell membrane permeability to K via muscarinic receptor activation, while the adrenergic transmitter (NA) stimulates the Na-K pump via β-adrenergic receptor activation.

References: Nishiyama, A. (1980), J. Physiol., 305, 95p.

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THE UPTAKE AND RELEASE OF EXCITATORY AMINO ACIDS IN THE CRAYFISH MUSCLE. TAKEUCHI, A., ONODERA, K. and KAWAGOE, R. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo, Tokyo 113

The abdominal slow flexor muscle of the crayfish was incubated with Harreveld's solution containing 0.1 mM or 0.5 mM L-glutamic-2,3,3,4,4-d_5-acid. After 30 minutes of washing with normal solution, the release of endogenous glutamic acid, glutamic-d_5 acid and aspartic acid into the bath fluid was measured with mass fragmentography using GC-MS. Glutamate-d_5 was probably taken up into the nerve terminal and/or the glial cells and was released during the resting period. The amount of resting release of glutamate-d_5 was approximately the same as that of endogenous glutamate released into the bathing solution. The amount of endogenous glutamate released was increased by 20 Hz nerve stimulation, but a significant increase of glutamate-d_5 release was not observed. Therefore, the glutamic acid that was taken up into the neural elements was not likely to enter a readily releasable transmitter pool. The application of 50 mM KCl solution induced a considerable increase in glutamate-d_5 release. Since such an increase of aspartic acid was not produced by the nerve stimulation, the high K solution causes a release of amino acids from some parts other than neural elements, in addition to a release of glutamate from a transmitter pool.

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INHIBITION PROCESS OF AXOPLASMIC TRANSPORT BY COLCHICINE IN CULTURED NERVE CELLS. HORIE, H., MAEDA, T*, and TAKENAKA, T. Dept. of Physiol., Sch. of Med., Yokohama City Univ., Minamiku, Yokohama 232

The number of transported particles passing through one region decreased after exposure to colchicine or vinblastine in concentrations greater than $1x10^{-7}M$, but velocities of transported particles were not affected by the agents. After treatment of the agents swelling occurred at regions where particles stopped or slowed down in normal conditions. The intervals of the distances between swollen regions were 5-25μm and transported particles finally stopped in the swollen regions. After 1 hour treatment of $1x10^{-5}M$ colchicine, electron micrographs showed many organelles but no microtubules in the swollen region. It is thought from these results that the disruption of microtubules in neurites might occur at the ends of microtubles and be followed by the swelling in neurites and the block of the transport of particles, but the morphological change itself might not have an affect on the transport of particles.

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FAST AXONAL TRANSPORT OF ACETYLCHOLINE AND PROTEIN IN A CHOLINERGIC NEURON OF APLYSIA. KOIKE, H. and OHTA, K. Dept. of Neurophysiology, Tokyo Metropolitan Institute for Neurosciences. Fuchu City, Tokyo 183

We injected [^{14}C] acetylcholine (ACh) and [^{3}H] leucine simultaneously into a neuron soma of Aplysia kurodai, and studied the axonal transport of ACh and protein in a cholinergic(R2) or a non-cholinergic(R1) neuron. Following several hours of culturing after the injection, the axon was sectioned to 1 mm length, homogenized and centrifuged (140,000g for 1 hour) to detect the intra-axonal movements of radioactive substances. The membrane protein and bound ACh moved in the cholinergic neuron's axon somatofugally at the maximum speeds of 3 and 2 mm/hr respectively at 25°C. Soluble protein, amino acid and soluble ACh diffused in the axon, but a portion of soluble ACh was actively transported in the axon. The axonal transport of ACh, both bound and soluble forms, was only observed in a cholinergic neuron but not in a non-cholinergic neuron. It was suppressed effectively by cytochalasin B (20 μg/ml in culturing solution), but not by colchicine (1 mM). On the contrary, the axonal transport of protein was suppressed by colchicine but not by cytochalasin B. It is suggested that the transmitter specific axonal transport of ACh is different in mechanism from that of protein.

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HORMONAL REGULATION AND SUBCELLULAR LOCALIZATION OF PROTEINASE F IN THE MOUSE SUBMANDIBULAR GLAND. HOSOI, K., TANAKA, I. and UEHA, T. Dept. of Oral Physiology, Josai Dental University, Sakado-shi, Saitama 350-02

The submandibular gland (SMG) of the mouse is known to contain proteinase A, D, and P-esterase, all of which are synthesized under the control of both androgens and thyroxines. Recently we found the presence of another species of esteroproteinase (a trypsin-like serine proteinase) in females in an appreciably high concentration and designated it as "proteinase F". Both the amount and the in vivo synthetic rate of proteinase F in males were only about 1/10 of those in females. However, they increased with castration and decreased again upon injection of androgens. Thus, proteinase F is the first known proteinase in the SMG the synthesis of which is inhibited by androgen. By electron microscopy using the ferritin-labeled-antibody technique, proteinase F was found to be exclusively localized in the smaller granules of granular convoluted tubule (GCT) cells with strong basal striations and in lesser amounts in the larger granules of GCT cells without basal striations. The results indicate that proteinase F should be an advantageous probe for the study of the origin and/or development of GCT cells in the SMG.

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STIMULATION OF THE OXYGEN UPTAKE BY SUBSTANCE P AND ACETYLCHOLINE IN RAT SUBMANDIBULAR SLICES. NAKANO, K., MORITA, M., KOMABAYASHI, T. AND TSUBOI, M. Komazawa Women's Coll., Setagaya, Tokyo and Dept. of Pharmacol., Hachioji, Tokyo

Substance P and acetylcholine significantly stimulated the oxygen uptake. When Ca was removed from the medium or the tissues were treated with 2 mM EGTA for 60 min, the oxygen uptake induced by substance P and acetylcholine considerably decreased. The oxygen uptake of Ca-deficient tissues due to acetylcholine was recovered by the addition of 2.5 mM Ca, while the response of substance P was recovered by the addition of 5 mM Ca. The oxygen uptake stimulated by acetylcholine was significantly inhibited by Ca inhibitors, diltiazem, verapamil and La. On the other hand, the oxygen uptake stimulated by substance P was significantly inhibited by diltiazem and La, but not by verapamil. The response of acetylcholine and substance P was not inhibited by calmodulin inhibitors, trifluoperazine, chlorpromazine and W-7. These results suggest that the increase in the oxygen uptake seen with acetylcholine and substance P is dependent on the presence of Ca, but is not concerned with calmodulin.

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EFFECTS OF K AND PILOCARPINE ON THE RELEASE OF PROTEIN-LIKE SUBSTANCES IN DOG SUBMANDIBULAR SLICES. KOMABAYASHI, T., NAKANO, K., SAKAMOTO, S., KAWAJIRI, Y. AND TSUBOI, M. Dept. of Pharmacol., Tokyo Coll. Pharm., Hachioji, Tokyo and Komazawa Women's Coll., Setagaya, Tokyo

Pilocarpine significantly stimulated the release of amylase, sialic acid and protein at 10^{-6}-10^{-4}M, and K also significantly stimulated the release at 75 mM K, The release of amylase, sialic acid and protein due to 10 μM pilocarpine and that due to 75 mM K both increased with the passage of time. When Ca-deficient tissue was incubated in the Ca-free medium, the effects of pilocarpine and K were inhibited. The amylase, sialic acid and protein release induced by pilocarpine and K was considerably inhibited by Ca inhibitors, La, diltiazem and verapamil. In particular, La inhibited the effects of these agents more strongly than diltiazem and verapamil. Also, the response of pilocarpine and K was significantly inhibited by calmodulin inhibitors, trifluoperazine, prenylamine and W-7. These results suggest that the release of protein-like substances induced by pilocarpine and K is dependent on the presence of Ca and closely related to calmodulin in the tissue.

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REGULATION OF FORMATION AND MAINTENANCE OF INTERCELLULAR COMMUNICATION. 1. EFFECT OF TUMOR PROMOTERS AND CYCLIC AMP. KANNO, Y., SHIBA, Y., ENOMOTO, T., SASAKI, Y., SASTRODIHARDJO, S. AND YAMASAKI, H. Dept. of Physiology, Hiroshima University School of Dentistry, Hiroshima 734 and I.A.R.C., Lyon, Cedex 02, France

It has been demonstrated that 12-O-tetradecanoylphorbol-13-acetate (TPA) and related tumor promoters reversibly inhibit the formation and maintenance of electrical communication of cultured human epithelial cells (FL). In this study, we have investigated the effect of cyclic AMP(cAMP) on the communication and on the TPA-induced suppression of the communication. In the control, dibutyryl cyclic AMP (dbcAMP) and aminophylline do not change the communication of FL cells. These protect the inhibitory effect of TPA on the communication, but do not restore the communication suppressed already by TPA when added to the culture. 5'-AMP and Na-butyrate do not change the communication in the control nor protect the inhibitory effect of TPA. These results suggest that cAMP may play an important role in the TPA-induced suppression of the communication, as one of regulators of the communication.

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Measurement of membrane potential by the use of cyanine dye in isolated perfused rat pancreas

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Membrane potential of pancreatic acinar cell was measured by the use of cyanine dye, diS-$C_3(5)$. Rat pancreas was isolated and perfused, and absorbance was contineously measured with scanning organ spectrometer.
After pancreas was stained, extracellular potassium concentration was changed from 5.6mM to 10, 20, or 40mM. Perfusion with 2 mM K^+ solution caused an increase in absorbance, while perfusion with 10, 20, and 40mM K^+ solution caused decreases. There was a linea relation between $[K^+]_o$ and absorbance change. This relation was well consistent with that obtained by micro-electrode method. Insulin caused increase in absorbance. CCK-PZ at 0.2mu/ml caused remarcable increase in enzyme secretion and a small increase or no change in absorbance. These results suggest that insulin activates electrogenic Na^+ pump to cause hyperpolarization in membrane potential.

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Measurement of pyridine nucleotide fluorescence and field potentials from the guinea pig olfactory cortical slice.

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Tissue pyridine nucleotide (NADH) fluorescence was measured simultaneously with field potentials evoked by stimulation of lateral olfactory tract in guinea pig olfactory cortical slices, with the use of a micro-light guide connected to a fluorometer.

1. The tissue NADH fluorescence started to increase slowly and attained a plateau 6 to 10 min after the onset of hypoxia. The average value of the plateau was 18.5± 4.2% (n = 7) at 6 min of hypoxia.
2. With the start of hypoxia, the potentials disappeared before the NADH fluorescence attained the plateau. With the re-oxygenation following a hypoxic period of 6 min, both the NADH fluorescence and the potentials began to recover and reached the initial about 20 min after.
3. The relative amount of tissue ATP measured from the brain slices decreased by 26% (mean, n = 5) of the control at 6 min of hypoxia and recovered the initial value 20 min after the re-oxygenation.

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DIELECTRIC ANALYSIS OF OUTER MEMBRANE VESICLES PREPARED FROM RAT LIVER MITOCHONDRIA

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In our previous report, the dielectric behavior of intact mitochondria isolated from rat liver was successfully explained by a dielectric theory based on the 'double-shell' model on the assumption that the outer and the inner membranes both have very low conductivities compared with the outer medium. The assumption holds obviously for the inner membranes in view of their permeability but is dubious for the outer membranes. To prove the validity of the assumption for outer membranes, we examined the dielectric behavior of outer membrane vesicles (OMV) prepared from intact rat liver mitochondria by a modification of the method of Persons. The OMV suspended in 130mM KCl showed the Cole-Cole type dispersion having a characteristic frequency of 50 MHz. When volume fraction was given by non-electrical methods (combination of mitocrit and stereological analysis), the conductivity ratio of the membrane to the outer medium could be roughly estimated from the limiting conductivity of the dispersion to be less than 3×10^{-4}. Therefore, the assumption in question was also applicable to the outer membranes; the observed dielectric data of OMV could be analyzed according to the 'single-shell' model. The estimated membrane capacity was about 1 $\mu F/cm^2$.

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CONDUCTANCE INCREASE OF INTERCELLULAR CHANNEL INDUCED BY STEROID HORMONE APPLICATION. SUZUKI, K. Dept. of Physiology, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa, 259-11.

The electrical conductance between neighboring two cells in dissected salivary gland of Chironomus thummi could be known by measurement of electrical coupling ratio between adjacent cells. The observed coupling ratios in freshly dissected preparation distributed widely from high value ($V_2/V_1 \simeq 1.0$) to low one ($V_2/V_1 = 0.5$). Our previous study (Suzuki & Higashino, 1977) showed that corticosteroids revealed a protective action for gland cells against blocking effect brought about by decoupling agents. In the present experiment, hydrocortisone (10 µg/ml), dexamethasone (0.25 µg/ml) and 17-β estradiol (5ng/ml) were applied to gland cells, which showed initially low coupling ratio below 0.5. It was found that the ratio between those cells increased up to 1.0 within one hour. Furthermore, originally inpenetrable fluorescent dye (fluorescein M.W. 330), injected into one of the poorly coupled cells, could now be seen in the adjacent cell one hour after application of hydrocortisone. It is suggested that hydrocortisone, dexamethasone and estradiol open the gate for intercellular low-molecule channel.

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EFFECTS OF DIVALENT CATIONS ON ACETYLCHOLINE-EVOKED MEMBRANE POTENTIAL IN THE IONOPHORE A23187 TREATED MOUSE PANCREATIC ACINAR CELLS. IWATSUKI, N., NISHIYAMA, A. Dept. of Applied Physiology, The First Dept. of Physiology, Tohoku University School of Medicine, Sendai, 980.

Intracellular ionized calcium (Ca) plays a key role in stimulant-evoked membrane potential in pancreatic acinar cells. In this study we investigated an important role of extracellular divalent cations on acetylcholine (ACh)-evoked membrane depolarization in the pancreatic acinar cells treated with the ionophore A23187 which causes depletion of intracellular stored Ca. The ionophore A23187 in the absence of extracellular Ca abolished the short pulse of ACh-evoked membrane depolarization. The subsequent exposure of the ionophore A23187 treated tissue to a Ca- or Sr- containing solutions restored the ACh-evoked responses. Ba, Co or Mn had no restrative effects. Mn or Co suppresed the restrative effects of Ca or Sr. The equilibrium potential of ACh-evoked depolarization in the acini treated with the ionophore A23187 and subsequently superfused with Ca or Sr was similar to the value of -18 mV in the control. It is concluded that the short pulse of ACh-evoked membrane depolarization seems to be dependent on extracellular Ca in the acini where intracellular stored Ca is depleted.

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BINDING CONSTANTS OF Na^+ IONS FOR THE GRAMICIDIN CHANNEL. MONOI, H. Dept. of Physiology, Tohoku University School of Medicine, Sendai 980

The excess longitudinal relaxation rate (ΔR_1) of ^{23}Na ions in gramicidin-doped lecithin liposomes suspended in NaCl solutions was measured as a function of the ionic activity a at a radio frequency of 79.4 MHz. When $a/\Delta R_1$ is plotted against a, a sublinear relationship is obtained, which is difficult to explain by simple one-cation-occupiable model of the channel. An electrostatic calculation suggests that three Na^+ ions cannot simultaneously be loaded by the gramicidin channel even if the presence of an anion at each channel mouth is supposed. The two-cation-occupiable two-identical-site model leads to: for 25°C (10°C), first binding constant (K_1) = 13.7 ± 1.4 (13.7 ± 1.5), second binding constant (K_2) = 1.6 ± 0.2 (0.72 ± 0.12), and T_1 of bound ^{23}Na in single (T_1^{sing}) and double (T_1^{doub}) occupancy = 0.56 ± 0.03 (0.49 ± 0.03) and 0.27 ± 0.01 (0.17 ± 0.01), respectively; quadrupole coupling constants for single (χ^{sing}) and double (χ^{doub}) occupancy are 1.3 and 1.8, respectively, at 25°C, and χ^{doub} is at least 2.3 at 10°C. These data involve several aspects that are difficult to explain by simple two-site models. The relation $\chi^{doub} > \chi^{sing}$ cannot be attributed to the electrostatic interaction between bound cations. These difficulties virtually vanish if the formation of an ion pair at the mouth is included in doubly loaded channels. Estimates of K_1, T_1^{sing} and χ^{sing} remain (practically) the same in both models. (K, T_1, and χ are expressed in $molal^{-1}$, ms, and MHz.)

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ENERGY METABOLISM IN EXPERIMENTAL CEREBRAL ISCHEMIA OF RAT BRAIN BY TOPICAL MAGNETIC RESONANCE (TMR). WATARI, H., SHIGA, K., NISHINA, Y., NARUSE, S.*, KOIZUKA, I.* AND TAKADA, S.* Department of Molecular Physiology, National Institute for Physiological Sciences, Okazaki 444, Japan.

TMR has been deviced with a wide bore superconducting magnet, in which a magnetic focusing (1.5 cm) is created by using profile coil. ^{31}P NMR spectra can be acquired from a selected deep place within living animals, such as brain. Wistar rats weighing about 200g were anesthetized by sodium pentobarbital (35 mg/kg). Preischemic period was obtained by coagulation of vertebral arteries. Then ischemic period followed by ligation of common carotid arteries, and thus experimental cerebral ischemia was induced with the four-vessel occulusion. The common carotid arteries were untied after this 30-minute period so as to get recirculation period. Within 4 minutes after the ischemia, ATP and phosphocreatine decreased with concomitant increase of inorganic phosphate (Pi), and pH had decreased from the normal value of 7.27 to a value of 6.52. As soon as the circulation was restored following the 30 minutes period of ischemia, Pi was rapidly declined and pH returned to that of preischemic period. Within 30 minutes after the recirculation, the ^{31}P NMR spectrum recovered to almost normal shape though ATP was slightly lower than that of the preischemic period. About 3 hours after the recirculation, the ^{31}P NMR spectrum was identical with that of the preischemic period.

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ON THE INFORMATION PROCESSING SYSTEM AND REGULATION OF FUNCTIONS IN MITOCHONDRIA. HATASE, O., TOKUDA, M., ITANO, T., DOI, A., TAOKA, T., OHMURA, Y., and NISIDA, I. Dept. of Physiology, KAGAWA Medical School, Miki-Cho, Kagawa 761-07

The cellular functions and structure are regulated by calmodulin (CaM) in the presence of calcium (Ca). The CaM-Ca system controls the enzymatic activities, ion concentrations, and structure of cytoskeleton in responce to the humoral informations. We communicated the presence of CaM in the mitochondrial matrix (Hatase et al. B.B.R.C. 104 [2], 673, 1982), that suggested the presence and roles of the simular regulatory mechanism in the mitochondrial matrix. In the present communication, we shall report the evidences of the presence of the mitochondrial regulatory system: In beef heart mitochondria there are CaM and its binding protein(s) that specifically binds to CaM and inhibits the CaM-stimulatory activity on phosphodiesterase in the presence of Ca. The heat-stable CaM-binding protein (BP) was purified by CaM-Sepharose 4B affinity chromatography, and CaM was purified by CAPP-Sepharose 4B affinity chromatography as well. The purified BP showed strong inhibitory effects on the CaM-stimulation of phosphodiesterase. One micro gram of BP (10 times amount of CaM) was required to show the 50% inhibition of the CaM (100 ng)-stimulation. The molecular weight of the purified BP was 61000 determined by gel filtration and SDS-polyacrylamide gel electrophoresis. The roles and regulatory mechanism of mitochondrial CaM-BP system will be discussed.

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THE CIRCADIAN RHYTHM OF THE LOCOMOTOR BEHAVIOR IN A POPULATION OF PARAMECIUM AND ITS SIGNIFICANCE ON THE ADAPTIVE STRATEGY. Hasegawa, K. and Tanakadate, A. Dept. of Physiol., Sch. of Med., Kitasato Univ., Sagamihara, Kanagawa 228

The circadian locomotor behavior of a Paramecium population was described in terms of swimming speed (V) and turning frequency (frequency of abrupt change of moving direction=F_{AR}), using a fully computerized video system. The two components were examined by statistically analysing widths and shapes of electric signals, which were generated when magnified-video-images of the specimens traversed beneath a photocell placed on a screen of a video monitor. It appeared that the specimens swam fast and unidirectionally during the day, while they swam slowly and turned frequently at night in a light-dark cycle (LD). This oscillatory pattern was sustained in DD, where the fluctuation of F_{AR} was a dominant characteristic, with amplitudes about half of those in LD. When either of the two components increased, the other decreased. Light enhanced V and diminished F_{AR}. The time structure of "random walk" of Paramecium behavior was examined by testing histograms of interval times between the consecutive signals. Joint analyses of the two components and interval histograms indicated that circadian organization of the two components of individual specimens resulted in accumulation/dispersal behavior of the population, which was attributed to circadian alternation of electric properties of the cell membrane of individual specimens.

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COLLAGEN GEL EMBEDDED CULTURE OF MOUSE MAMMARY EPITHELIAL CELLS. ENAMI, J., KAWAMURA*, K., KOEZUKA*, M. and KOGA, M. Dept. Physiol., Dokkyo Univ. Sch. Med., Mibu, Tochigi 321-02.

Normal mammary epithelial cells and mammary tumor cells of the mouse were cultured embedded in the collagen gel matrix. When the epithelial cells were cultured in the presence of insulin, epidermal growth factor (EGF) or mammary fibroblast-conditioned medium supplemented to the basal medium (DME + 10% fetal bovine serum), three-dimensional branched growth was observed. Furthermore, colonies of rapidly growing cells were occasionally seen in cultures of mammary tumor cells. Normal mammary epithelial cells can also be grown under serum-free conditions. EGF or conditioned medium factor supplemented to the basal medium (a 1 : 1 mixture of Ham's F12 and DME + bovine serum albumin + insulin + transferrin) supported the formation of hollow structures resembling alveolar/ductal structures. By using the current collagen gel embedded culture technique, long-term cultivation including passaging has also been possible.

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EFFECT OF PYRUVATE ON PROLIFERATION OF ADULT RAT HEPATOCYTES IN PRIMARY CULTURE. HASEGAWA, K., WATANABE, K. and KOGA, M. Dept. Physiol. Dokkyo Univ. Sch. Med., Mibu, Tochigi 321-02.

We have succeeded in inducing DNA synthesis and mitosis in primary cultures of adult rat hepatocytes under serum-free conditions. Proliferating hepatocytes maintained liver specific functions such as ornithine carbamoyl transferase and tyrosine aminotransferase for 6 days. Under our serum-free culture system, fibronectin, Trasylol®, pyruvate, proline, insulin, glucagon or epidermal growth factor and low concentration of Ca^{2+} were essential for hepatocyte proliferation. Among these factors, pyruvate was necessary for survival of hepatocytes and enhancement of DNA synthesis and mitosis. In high pyruvate (40 mM) medium supplemented with insulin and glucagon, 2- to 6-fold increase in DNA synthesis was observed as compared with that in low pyruvate (10 mM) medium. RNA and protein synthesis increased prior to DNA synthesis. Both of RNA and protein synthesis in high pyruvate medium were 2-fold over those in low pyruvate medium. These results suggest that a high concentration of pyruvate stimulates DNA synthesis and mitosis by increasing RNA and protein synthesis observed in prereplicative period.

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BIOSYNTHESIS OF TRANSFERRIN IN RAT LIVER. NAKADA, H., AND TASHIRO, Y. Dept. of Physiology, Kansai Medical University, Moriguchi, Osaka 570

Glycosylation of transferrin was investigated in vivo by using antibody monospecific for rat serum transferrin. Most of the intracellular transferrin (95%) was endo H sensitive, whereas serum transferrin was completely resistant to it. Fractionation of the total microsomes has revealed that the intracellular transferrin immunoprecipitated from the rough and smooth microsomes and the Golgi heavy subfraction are all endo H sensitive and most of the endo H sensitive oligosaccharides were eluted to the position corresponding to $Man_8GlcNAc$ on high resolution Bio-Gel chromatography. Endo H resistant form was first detected in the intermediate Golgi subfraction and mostly in the light counterpart, most of which were sensitive to neuraminidase. These results suggest that the major oligosaccharide form of intracellular transferrin in the course of intracellular transport from ER to Golgi apparatus is $Man_8GlcNAc_2$ and that the late processing of high mannose type oligosaccharide chains of the intracellular transferrin and the subsequent addition of terminal sugars to them are performed in the trans-Golgi region just before secretion.

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PRODUCTION OF PLASMINOGEN ACTIVATOR IN A MELANOMA CELL LINE. MATSUO, O., SAKAI, T., KANIIKE, K., NISHIDA, Y*., AKAZAWA, K*. and MIHARA, H*.
Dept. of Physiology, Kinki University School of Medicine, Sayama 589, and *Dept. of Physiology, Miyazaki Medical College, Kiyotake 889-16.

The plasminogen activator (PA) produced by melanoma cell lines has a higher affinity for fibrin and can digest fibrin which is the major protein of thromboemboli. Morphologically, the present cells had no specific features of shape in either a sparse or monolayered state. The present study revealed that PA was produced during cell growth. The production rate was rather constant and the production was proportional to the cell number. The greater the cell number, the stronger was the PA activity. As to intracellular PA, only when the number of cells became sufficiently large, was intracellular PA detected. The intracellular PA activity was proportional to the number of cells. However, the activity of the intracellular PA was usually weaker than that of the extracellular (medium) PA. Thus, during the cell growth of melanoma cell line (Bowes), intracellular PA is secreted or released into the medium, maintaining intracellular PA low.

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EFFECTS OF HUMORAL FACTOR FROM HUMAN TONSIL ON DIFFERENTIATION OF LYMPHOID CELLS KINOSHITA, Y., KIMURA, S., KOMANO, Y. and YAMAGA, Y. Dept. of Physiology and Otolaryngology, Osaka City Univ. Med. Sch., Abeno-ku, Osaka

Humoral factors(TonHF) were prepared from human tonsils according to the method for thymus humoral factor(THF). Lymphoid cells at various stages in their differentiation and maturation pathway existed in tonsil. Effect of those factors on differentiation and maturation of human tonsillar lymphoid cells were examined. For examining the effect, responding capacity to lectin stimulation and binding ability to sheep erythrocytes(rosette-forming capacity) were tested. Soybean-lectin(SBL) was assumed to react to precursor T cells(PTC), while concanavalin A(Con A) caused blastoid transformation of T cells(thymus-dependent lymphocytes). Rosette-forming capacity was mainly found in T cells. Pretreatment of the lymphoid cells with TonHF induced an enhancement of SBL-response. However, changes in rosette-formation and responses to Con A couldn't be induced by the factor. On the other hand, pretreatment of the cells with THF induced an increment of Con A-response and number of rosette-forming cells, while responsiveness to SBL stimulation was reduced. These results suggested that TonHF induced a change of undifferentiated cells to PTC, while THF caused differetiation of PTC to T cells. Mechanism of differentiation and maturation in the lymphoid cells seemed to be scrutinized by those factors.

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INHIBITORY EFFECTS OF MONOSACCHARIDES ON ROSETTE FORMATION BETWEEN RAT THYMUS LYMPHOCYTES AND GUINEA PIG ERYTHROCYTES. OKA, H., KIMURA, S. and KINOSHITA, Y.
Dept.of Physiology, Osaka City University Medical School, Abeno-ku, Osaka

High proportions of lymphocytes from rat thymus participate in rosette formation (RF) with guinea pig erythrocytes only in the presence of non-heated fetal calf serum (FCS). FCS contains heat stable and labile factors.

After neuraminidase treatment of rat thymus lymphocytes, the cells participating in RF increased, especially in thymus medullary lymphocytes which were collected after cortisol injection. This indicates that some of receptors of heat stable factor are masked by sialic acid. RF was inhibited by addition of monosaccharides such as L-Fucose and D-Mannose. However, RF was not inhibited by these sugars when thymus lymphocytes were precoated with FCS. Furthermore, RF activity was decreased when FCS was adsorbed with Mannose-Sepharose column prior to mixing FCS with lymphocytes and erythrocytes.

From our data, it appears that the site of inhibition of Mannose in the RF system is heat stable factor of FCS, and that heat stable factor binds with D-Mannose on lymphocyte surface.

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ON THE PLASMA MEMBRANE PROTEINS OF NEW L-N CELLS SPONTANEOUSLY TRANSFORMED FROM L929 CELLS. DOI, A., TOKUDA, M., TAOKA, T., ITANO, T., HATASE, O. and NISIDA, I. Dept. of Physiology, Kagawa Medical School, Miki-cho, Kagawa 761-07

The L-N cells spontaneously transformed in vitro after 300 generations, that lost serum dependency for proliferation. The generation time of the L-N cells was shorter than that of the original strain (L929); 18 h and 22 h, respectively. The changes in the pattern of cell size distribution under synchronized condition confirmed the results. The electron microscopical studies revealed the structure of cell surface of the L-N cells were different from that of the L929 cells. The plasma membranes of the L-N and L929 cells were prepared by the sucrose density gradient centrifugation from the asynchronized and synchronized populations in the growing and resting media. SDS containing polyacrylamide gel electrophoresis revealed the remarkable decrease in the 80-100K polypeptides group in the L-N cells compared with the L929 cells in G_1 phase. Amino acid analysis of the conditioned media showed that the remarkable increase of proline and glycine in the L-N cell system compared with the L929 cell system.

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HISTOCHEMICAL AND ULTRASTRUCTURAL OBSERVATION ON EPITHELIA OF A YELLOWTAIL EXPOSED TO SEA BLOOM. TOYOSHIMA,T and MURAKAMI,T.H, Dept. of Biol., Kagawa Med. Sch., Kagawa 761-07

The effects of cultured sea bloom*(Chattonella antiauia(HADA) ONO)*, on gill lamellae of the yellowtail*(Seriola quinqueradiata)*, were investigated with a scanning and transmission electron microscope and histochemical methods. The fish was exposed in plankton suspension (cell density was 3,000 cell/ml), and in different oxygen contents. The fish died after 34 min. in low oxygen content and died after 115 min. in high oxygen content. Histochemically, it was observed that fish were exposed in low oxygen content, almost loss of mucous cells on the afferent and efferent ridge of primary lamella, in contrast, in moderate and high oxygen content degeneration of mucous cells only on afferent ridge. Electronmicroscopically, the suface of the efferent ridge exposed to sea bloom, secretory granules of mucous cells disappeared and porous epithelia were observed, while chloride cells appeared on surface of the interlamella space, and edema formation was observed in secondary lamella particulary in its basement.

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DIFFERENTIATION OF Na ACTIVE TRANSPORT SYSTEM DURING METAMORPHOSIS THROUGH TADPOLE SKIN.
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Though sodium was actively transported from the epidermal side to the dermal side through the adult bullfrog skin, there was no Na active transport through the tadpole skin of the earier stage. Potential difference across the skin (PD) and short circuit current (SCC) appeared and exponentially increased after stage XX of tadpole. Cadmium-induced increase as well as amiloride-increased decrease in PD and scc were also observed in accordance with the developments of PD and SCC through the control tadpole skin. The skin resistance (Rm) gradually increased on developing the stage of tadpole. Amiloride increased the percent increase in Rm after stage XX, however, effect of Cd was quite different. Cadmium increased Rm (%) before the stage XXI, on the contrary, it decreased Rm (%) after stage XXV. The resistance to the passive ions (Rs) was increased by cadmium throughout the whole stage. It is postulated that Rs channel in the skin exists already in the earier stage, but Na channel relating the active Na transport develops after stage XXI.

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CHANGES IN THE INTRACELLULAR Ca^{2+} AND K^+ CONCENTRATIONS DURING THE RECEPTOR-MEDIATED HYPERPOLARIZING RESPONSES IN THE FIBROBLASTIC L CELLS.
*OIKI, S, **UEDA, S. and OKADA, Y. Dept. of Physiology, *Dept. of Anesthesiology and **Dept. of Internal Medicine, Fac. of Medicine, Kyoto University, Kyoto 606

Mouse fibroblastic L cells respond with slow hyperpolarizations to ATP, β-lipoprotein and complement via each receptor systems. By means of Ca^{2+}- and K^+-selective microelectrodes (made with Simon's neutral carrier and Corning 477317, respectively), the intracellular concentrations of free Ca^{2+} and K^+ ions ($[Ca]_i$ and $[K]_i$) were measured upon the application of these chemicals. The receptor-mediated hyperpolarizing response coincided with a transient increase in $[Ca]_i$ up to 1.2 μM from the resting level (about 0.5 μM). During the hyperpolarizing responses, the $[K]_i$ value gradually decreased from the original level (about 130 mM). When the hyperpolarizing responses finished, the $[K]_i$ value reached a lower steady level (about 110 mM). The membrane potential level was quantitatively correlated with the $[Ca]_i$ level and the rate of the $[K]_i$ decrease. These observations provide direct evidence for the concept that the receptor-mediated hyperpolarizing responses are cause by the stimulation of Ca^{2+}-activated K^+ conductance.

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ISOLATION OF CANINE (Na^+, K^+)ATPase α AND β SUBUNITS.
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The α and β subunits of (Na^+, K^+)ATPase from dog kidney were purified by lectin-Sepharose in addition to preparative SDS-PAGE. The amino acid composition of the α subunit was much the same as the data from Kyte (Kyte, J. 1972, J. Biol. Chem. 247,7642-7649). However the amino acid and carbohydrate compositions of the β subunit were different from his data. The subunit contained few of histidine and very large amount of carbohydrate. Antibody against each subunit reacted specifically to protease fragmented α subunit and neuraminidase treated β subunit as well as the intact subunit, respectively. These results indicate that the α and β subunits were highly purified.

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ION TRANSPORT IN AMPHIBIAN RENAL TUBULAR CELLS DETERMINED BY X-RAY MICROANALYSIS.
S. Sasaki, I. Nakagaki, Y. Imai, M. Fujimoto, G. Kuramochi*and T. Hoshi.* Dept. of Physiol., Osaka Med. Coll., Takatsuki, Osaka 569 and *Dept. of Physiol., Facult. of Med., Tokyo Univ., Tokyo, Japan.

The electron probe X-ray microanalysis of bullfrog and triturus renal tubular cells were carried out. The energy-dispersive X-ray spectra from the frozen ultrathin sections of bullfrog nephron showed high peaks for K and P, and low peaks for Na and Cl in the cytoplasm, nucleus and mitochondria, whereas they showed relatively high peaks for P, S, K, Na and Cl, and a low peak for Ca in the lysosomes of proximal tubular cells. The effects of ouabain (10^{-4} M) in proximal tubules was examined. By the treatment of ouabain the Na and Cl concentrations increased and the K concentration decreased in the cytoplasm, while in lysosomes the Na and Cl concentrations decreased and the K concentration increased. The role of lysosome in proximal tubular transport of ion, water and other organic substances was discussed. X-ray microanalysis of triturus renal tubular cells was also performed. The spectra from cytoplasm showed, in addition to high K and P, relatively high peaks for Na and Cl in early distal tubular cells while low peaks for Na and Cl in proximal tubular cells. Load of L-alanine (10^{-2} M) resulted in the increase of Na and Cl in the cytoplasm of proximal cells. Exposure to furosemide (10^{-5} M) resulted in the decrease of Cl in the cytoplasm of early distal cells.

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ANALYSIS OF RENAL TUBULAR HANDLING OF CALCIUM WITH ION-SELECTIVE MICROELECTRODES.
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Calcium concentration ratios of tubule fluid to serum (ultrafiltrable ionized form, (TF/UF), were determined in normal bullfrog nephrons with Ca-selective double-barreled microelectrodes. The mean (TF/UF) ratio was 1.23 in the proximal convoluted tubule with a lumen-negative PD of -6.3 mV. There was a significant correleation between the individual ratios of (TF/UF) and the luminal PDs ($n=27$, $r=0.394$, $p<0.05$), although a slight deviation was recognized between the calculated Ca-equilibrium potential and the actually measured transtubular PD across the proximal segment. A downhill gradient of transtubular net electrochemical gradient for Ca, which is favorable to the movement of Ca from the peritubular to the luminal fluids, would suggest a possibility of Ca backflux through the intercellular space along with an active Ca reabsorption across the proximal tubule. In the early distal tubule, the (TF/UF) ratio fell to 0.51 with a reversal of luminal PD (+11 mV). In the final urine, the (U/UF) ratio further fell to 0.15. Using the total chemical concentration in the urine, the ratio was shown to be 0.25. This suggests that Ca is reabsorbed progressively along the distal nephron segment, and a complex formation of Ca with anions, such as phosphate, would not reduce the overall Ca reabsorption. Thus, it is concluded that the filtered Ca is actively reabsorbed along the most of nephron segments.

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INTRACELLULAR Cl^- ACTIVITY OF THE PROXIMAL TUBULE OF TRITURUS KIDNEY: THE DEPENDENCE ON EXTRACELLULAR IONIC COMPOSITION AND THE TRANSMEMBRANE POTENTIAL.
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Intracellular Cl^- activity (a^i_{Cl}) was measured with double-barreled Cl^- selective microelectrodes in Triturus proximal tubule, and the effects of ionic composition of the perfusion solutions and the cell membrane potentials were studied. In the absence of HCO_3^-, a^i_{Cl} averaged 22.4 ± 0.4 mM, the value being 3.8 times that expected from the Nernst equation. Increases in peritubular HCO_3^- concentration without changing pH resulted in drastic decreases in a^i_{Cl}. Addition of HCO_3^- only to the luminal fluid had no effect. Elimination of Na^+ from the luminal fluid caused no immediate changes in a^i_{Cl}, but long-lasting perfusions with a Na^+-free solution resulted in a gradual decrease in a^i_{Cl}. Lowering or raising intralumial pH rapidly increased or decreased a^i_{Cl}, respectively. The alanine-induced depolarization of the cell membranes exceeding 15 - 20 mV rapidly increased a^i_{Cl}, and the increase was proportional to the driving force for Cl^-. The results obtained suggest the presence of a Cl^-/OH^- antiport mechanism in the luminal membrane and a Cl^-/HCO_3^- exchange mechanism and voltage-dependent rectifying Cl^- pathways in the peritubular membrane.

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ION TRANSPORT DEPENDENT OXYGEN CONSUMPTION OF THE EARLY DISTAL TUBULE OF TRITURUS KIDNEY. KURAMOCHI, G., HOSHI, T*., KAWAHARA, K*. and MIURA. M*. 2nd Dept. of Int. Med., Niigata Univ. Sch. Med., Niigata, *Dept. of Physiol., Univ. of Tokyo, Bunkyo-ku, Tokyo

The early distal tubule (eDT) of amphibian kidney is a unique portion of nephron where Cl^- is actively transported. In attempts to know relation of oxygen consumption to ion transport, medial loops (mainly eDT) and lateral convoluted tubules (mainly proximal tubules, PT) were dissected from Triturus kidneys and oxygen consumption (Qo_2) of these tissues were compared under various medium conditions using an oxygen electrode. Replacement of Cl^- with NO_3^- diminished Qo_2 of eDT by 48% but not affected that of PT. Elimination of Na^+ (choline substitution) inhibited Qo_2 of both eDT (42%) and PT (33%). Furosemide (1 mM) depressed Qo_2 of eDT (47%) only whereas ouabain (1 mM) inhibited Qo_2 of both eDT and PT (64% and 32%, respectively). Addition of Ba^{++} (1 mM) to the medium inhibited Qo_2 of eDT (25%) only without affecting Qo_2 of PT.

Electrophysiologically, generation of the Cl^- transport potential was found to absolutely require both Na^+ and K^+ in the luminal fluid and completely inhibited by luminally applied furosemide. Ba^{++} appeared to block secretory fluxes of K^+ into the lumen, thereby inhibit K^+-dependent NaCl transport. These data are consistent with the hypothesis that Cl^- is transported across the luminal membrane by a $2Cl^-/Na^+/K^+$ cotransport mechanism in the eDT.

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INFLUENCES OF PROSTAGLANDINS ON THE VASOPRESSIN-INDUCED OSMOTIC WATER FLOW OF THE TOAD BLADDER F.marumo, H.Shimada and H.Sakamoto, Dept.Med., Kitasato Univ.Sch.Med., Sagamihara, Kanagawa

Influences of prostaglandins on the vasopressin(Vp)-induced water flow across the toad bladder were studied. Exogenous PGE_1 and PGE_2 suppressed Vp(10mU/ml)-induced water flow from 10^{-8}M. Both 1-iodo-3-aminomethyl-5,6,7,8-tetrahydro-2-naphthol (ONO-3122), which increases enogenous PGH_2, and PGH_2 itself, significantly depressed Vp-induced osmotic water flow, while PGI_2 did not. These results suggest that PGH_2 and/or its metabolites inhibit Vp-induced water flow. Both di-homo-linolenic acid and arachidonic acid enhanced the inhibitory effect of ONO-3122. Indomethacin enhanced both Vp- and cyclic AMP(5mM)-induced water flow, and the half maximum activation doses were approx. 5×10^{-9} and 5×10^{-7}M, respectively. Also, the half maximum inhibition doses of ONO-3122 on Vp effect was 1-2 order lower than cyclic AMP. These results suggest that endogenous PGE_2 depresses the Vp-induced osmotic water flow, by suppressing the adenylate cyclase - cyclic AMP system both before and after production of cyclic AMP. (E)-2-methyl-3-((4-(3-pyridylmethyl)phenyl))-2-propenoic acid sodium salt (OKY-1581) depressed Vp-induced water flow at 10^{-6}M, while activated it at 10^{-4} and 10^{-3}M. These results suggest that OKY-1581 inhibits thromboxane synthesis at low concentration, while it inhibits endoperoxidase at high concentration.

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NETWORK SIMULATION ON THE TRANSPORT SYSTEM OF THE EPITHERIAL CELL.
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Applying the network thermodynamics to the membrane transport system in biology, we can treat the transport phenomenon as a bond graph model in which various energy flows and the various energy power couplings exist. We constructed a bond graph model of the transport system on the gland cell. This model allows the volume flow and various ionic flows including the active transport by Na-K pump. From this bond graph model,we can obtain several transport equations and the equations of the rate of changes on force variable in the cell. Using the set of equations, we examined the behavior of the cell model with step by step computer simulation method. On the model, in steady state, sudden changes of ionic mobilities in the membrane caused the membrane potential changes(Hyperpolarization) and volume flow into the cell as observed in a real gland cell system.

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EFFECT OF AMILORIDE ON THE ELECTROCHEMICAL GRADIENT FOR K ION ACROSS THE MEMBRANES OF TURTLE BLADDER CELLS IN OPEN- AND SHORT-CIRCUITED CONDITIONS. SATAKE, N., MORIMOTO, Y., KOTERA, K. and FUJIMOTO, M. Dept. of Physiol., Osaka Med. Coll., Takatsuki, Osaka

To examine the cellular mechanism of the ion transport across the turtle bladder, the electrochemical gradient for K^+ across the membrane was investigated with K^+-selective double-barreled microelectrodes applied to the Ussing preparation. In normal Ringer bathing media, the transepithelial potential difference (PD) showed 57 mV (n=14, mucosal negative), and mucosal membrane potential (mEm) -7 mV (n=9, cell= 0 mV). The cellular K^+ activity was 80.9 mEq/L (n=19) with respect to 4.1 mEq/L for Ringer solution, and these values remained unchanged in both open-circuited (OC) and short-circuited (SC) conditions. In the latter condition, however, the mEm was elevated to 29 mV (n=8) and short-circuit current (Isc) was shown 34 μA (n=14). Thus, the potential profile, which was shown as "step" type in the OC condition, could reversibly be converted into "well" type in the SC condition. After administration of 10^{-4} M amiloride in the mucosal medium, the cellular K^+ activity increased to 87.9 mEq/L (n=17) and the PD markedly decreased to -17 mV with a dramatic rise of mEm to 56 mV. The Isc was also lowered to -2 μA (n=15) and mEm elevated to 42 mV (n=5) in the SC condition. These data indicate that the potential jump across the mucosal membrane is amiloride-sensitive and it approaches the K^+ equilibrium potential after amiloride administration.

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EFFECTS OF $[K]_o$ ON THE STIMULATION OF GLUCOSE INFLUX BY INSULIN IN FROG SKELETAL MUSCLE. MARUNAKA, Y., KITASATO, H. and MURAYAMA, K. Department of Physiology, Shiga University of Medical Science, Ohtsu, Shiga 520-21

We studied the glucose transport in paired sartorii of *Rana catesbeiana*, using 3-O-methyl-D-glucose (3-O-MG), non-metabolized sugar. We had reported that 3-O-MG influxes were consisted with Na-dependent and Na-independent components; the former was insulin-sensitive and the latter was insulin-insensitive. In the present experiments, we studied effects of $[K]_o$ on the stimulation of the Na-dependent 3-O-MG influx by insulin. Insulin stimulated 3-O-MG influx at various external K concentrations (0-10mM). The stimulating effect of insulin on 3-O-MG influx decreased with a rise of the external K concentration. An addition of insulin brought about hyperpolarization and an increase in ^{22}Na influx. With a rise of the external K concentration, both the extent of hyperpolarization and the magnitude of the increase in ^{22}Na influx induced by insulin also diminished in parallel with a change in response of 3-O-MG influx to insulin. From these results, it is suggested that the diminution of insulin action on 3-O-MG influx caused by a rise of the external K concentration would be partly due to the decrease of the extent of insulin-induced hyperpolarization resulting from the diminution of electrogenicity of electrogenic Na-K pump.

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PROTON NUCLEAR MAGNETIC RESONANCE STUDY OF LACTATE TRANSPORT THROUGH MUSCLE MEMBRANE. SEO, Y., YOSHIZAKI, K., NISHIKAWA, H. and MORIMOTO, T. Dept. of Physiology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602

High-resolution proton magnetic resonance (^1H-NMR) spectra of intact frog muscle, obtained by the selective saturation of water signal, showed resonances from lactate, carnosine and other compounds. This technique was used to monitor the changes in lactate content estimated from the area of lactate signal and in intracellular pH estimated from the chemical shift of C_2-H of carnosine.

The isolated sartorius muscle of bullfrog was stimulated electrically under anaerobic condition. The intracellular lactate concentration was determined by ^1H-NMR and the efflux of lactate was determined from the changes in the lactate content of the incubation medium at various pH values. The efflux of lactate increased approximately linearly with the concentration difference of lactate up to about 30 mmoles per liter of cell water. The permeability coefficient of the muscle membrane for lactate was increased as the extracellular pH was increased. The permeability coefficient was 5.5 ± 0.5, 9.1 ± 0.7 and $10.6 \pm 0.6 \times 10^{-3} \cdot min^{-1}$ at the extracellular pH value of 6.4, 7.0 and 7.6, respectively. Based on the results, the mechanism of lactate efflux was discussed.

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SALIVARY SECRETION AND ATP METABOLISM IN DOG UNDER THE CONDITION OF LOW Na^+ PERFUSION(USING NMR). *MURAKAMI,M.,**SEO,Y.,***WATARI,H.,*NAKAHARI,T.,*MICHIGAMI,M.,*MORI,H. AND *IMAI,Y. *Dept.of Physiol.,Osaka medical col.,Takatsuki 569,**Dept. of Physiol.Kyoto Preferectual University of Medicine, Kyoto 604 and *** Dept.of Molecular Physiol.,National Inst. of Physiol. Sciences,Okazaki 444.

Phosphorus nuclear magnetic resonance(^{31}P-NMR) was used for the measment of time course for phosphorus energy metabolites in perfused canine submandibular glands. During control Krebs-Henseleit perfusion condition, ATP and creatine phosphate (CP) were 0.42 ± 0.11 mM(± S.D) and 0.62 ± 0.16 mM. After perfusion ceased, ATP and CP tissue levels decreased,ADP increased and pH decreased. Restarting perfusion led to return of tissue content of CP,ATP and pH control levels,while ADP decreased. Acetylcholine(Ach 10^{-6}M) administration induced salivary secretion,decreased levels of ATP,CP and pH, and increased ADP. When Na^+ was replaced complately by Li^+, resting levels of ATP and CP increased slightly. Under Na^+-depleted conditions, Ach induced only minimal salivary secretion and no change in ATP and CP levels. Reinstitution of Na^+ to the perfusion without added Ach caused a decreased in tissue levels of ATP and CP and slow salivary secretion.

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ELECTRICAL MEMBRANE RESPONSES TO GASTRIC ACID SECRETAGOGUES IN THE RAT PARIETAL CELLS IN MONOLAYER CULTURE.
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To carry out electrophysiological studies on parietal cells under a visual control, fragmented glandular stomach of neonatal rat was cultured. The epithelial cells migrated from the explant and formed a monolayer colony. The parietal-like, eosinophilic cells, which contained many large-sized vesicles within the cell, were found in the monolayer for the first 1-week period. Indirect immunofluorescence studies with anti-parietal cell antibodies (obtained from the patient of pernicious anemia) testified that these eosinophilic cells are originated from parietal cells. The resting potential was -10 to -30 mV in the cultured parietal cells. The cells responded with significant hyperpolarizations (up to -30 to -50 mV) to putative gastric acid secretagogues (gastrin, acetylcholine and histamine). The acetylcholine-evoked response was blocked by atropine but not by curare. Cimetidine (not pyrilamine) blocked the histamine response. Neither atropine nor cimetidine inhibited the gastrin response. Since the amplitudes of these responses were dependent on the extracellular K^+ (nor Na^+ and Cl^-), it is suggested that these receptor-mediated hyperpolarizations are caused by an increase in the K^+ permeability.

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ACID SECRETION IN THR GUINEA PIG COLONIC MUCOSA. SUZUKI, Y. and SUZUKI, T.* Dept. of Physiol., Yamagata Univ., Sch. of Med., Yamagata 990-23

The isolated guinea pig colon showed the ability to secrete acid into the mucosal bathing solution(at a rate of 1∿2μmoles/cm^2 hr) and alkaline into serosal bathing solution(at ∿50% of acid secretion rate), when the tissues were incubated with HCO_3^--free solution bubbled with 100% O_2. Changing the mucasal pH between 6.8∿7.8 or serosal pH between 7.0∿7.8 scarcely affected the acid secretion rate. Displacement of transepithelial potential difference(PD) by passing current across the tissue(±15mV from resting PD) did not cause any significant change in the acid secretion rate. Ouabain in the mucosal solution promptly(<1min) decreased the acid secretion rate(ED_{50}=5x10^{-6}M), and at the concentration of 10^{-4}∿10^{-5}M it abolished the acid secretion completely. Omission of K^+ from the mucosal bathing solution inhibited the acid secretion rate by ∿50%. Recently K^+-ATPase was reported to be isolated from the mucosal membrane of rabbit colon and shown to be similar to the gastric H^+-K^+ATPase(Gustin and Goodman, J. Biol. Chem.257:9629,1982). We concluded that similar H^+-K^+ATPase might be present and play a role in the mucosal membrane process of the acid secretion in the guinea pig colon.

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EFFECTS OF ADRENERGIC AGENTS ON ION TRANSPORT IN GUINEA PIG COLON. *ISHIDA, H. AND SUZUKI, Y. *Dept. of Surgery, and Dept. of Physiology, Yamagata Univ., Sch. of Med., Yamagata 990-23

We have examined the effects of adrenergic agents on short-circuit-current(Isc), transepithelial conductance(Gt), and fluid transport in the in vitro guinea pig colon. Noradrenaline(NAd, 10^{-5}M, serosal side) caused a transient increase in Isc with a slight increase in Gt in about 50% of the tissues studied, whereas in other 50% it induced a decrease in Isc with an increase in Gt. In most of the latter cases the responses of Isc were biphasic or triphasic, suggesting that the decreasing responses were mixed with the increasing responses described above. Both types of responses were reproduced by isoproterenol(10^{-5}M), but not by NAd+propranolol(10^{-5}M). Replacement of Na with choline in the mucosal solution scarcely affected these electrical responses. NAd was also shown to stimulate fluid absorption when the fluid absorption was determined gravimetrically, using everted sac preparations(-9±49:control to 78±40µl/g tissue w. wt. /30min, n=4). This response was also evoked by isoproterenol but not by NAd+propranolol. From the above data, we conclude that NAd cause three independent responses through β-adrenergic receptor(s) in this preparation.

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CHLORIDE TRANSPORT ACROSS THE SEAWATER EEL INTESTINE. ANDO, M. Lab. of Physiology, Faculty of Integrated Arts and Sciences, Hiroshima University, Naka-ku, Hiroshima 730

Chloride transport in the seawater eel intestine depends not only on mucosal Na^+ but also on mucosal K^+ (Ando, 1981). In the present study, it was also demonstrated that K^+ transport from mucosa to serosa was dependent on Cl^- and that the chloride-dependent K^+ flux was almost equal to the net Cl^- flux from mucosa to serosa, suggesting that there exists KCl cotransport system with coupling ratio of 1:1. In order to elucidate characteristics of the KCl cotransport system, effects of diuretics, furosemide (0.1 mM) and ethacrynic acid (0.5 mM), on the net Na^+, K^+ and Cl^- fluxes were examined simultaneously, and compared with the effects of them on NaCl cotransport system. These diuretics added to the mucosal fluid inhibited both Na^+ and Cl^- absorption at the same amount in normal Ringer solution, suggesting that these diuretics inhibits NaCl cotransport with coupling ratio of 1:1. After the mucosa was bathed with KCl Ringer solution, while the serosa was being bathed with normal Ringer solution, in this condition Cl^- was mainly absorbed through KCl cotransport system, 0.1 mM furosemide or 0.5 mM ethacrynic acid was added to the mucosal fluid. Furosemide inhibited both K^+ and Cl^- absorption but ethacrynic acid did not. Since ethacrynic acid does not inhibit KCl cotransport but does NaCl cotransport, it could be concluded that KCl cotransport system is distinct from NaCl cotransport system.

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INTERACTION OF GLYCYLGLYCINE AND Na^+ AT THE MUCOSAL BORDER OF GUINEA PIG SMALL INTESTINE. A NON-MUTUAL STIMULATION OF TRANSPORT. HIMUKAI, M., KAMEYAMA, A. AND HOSHI, T. Dept. of Physiol., Fac. of Med., Univ. of Tokyo, Tokyo-113

Sodium-dependence of glycylglycine(Gly-Gly) influx and stimulation of Na^+ transport by Gly-Gly were studied in everted sacs, sheet preparations and brush border membrane vesicles isolated from guinea pig ileum. Gly-Gly influx was found to be independent of the presence of Na^+, while Na^+ transport was stimulated by Gly-Gly as evidenced by increases in transmural potential difference, short-circuit current and Na^+ influx. The change in transmural potential difference induced by Gly-Gly was a saturable function Gly-Gly concentration, showing a Michaelis-Menten type relationship. The half saturation concentration for Gly-Gly estimated from the electrical data was nearly identical with that estimated from influx data. At a constant Gly-Gly concentration, the relationship between the increase in short-circuit current and Na^+ concentration was sigmoid. Kinetic analysis according Garay and Garrahan indicates that each Gly-Gly carrier has two equivalent non-interacting binding sites for Na^+, and that translocation of Na^+ occurs when the two Na^+ sites on the carrier loaded with Gly-Gly are occupied by Na^+. However, our results indicated that the resultant Na^+ flow is not capable of stimulating Gly-Gly translocation and Na^+ - Gly-Gly interaction is non-mutual.

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TESTS OF A CALCIUM HYPOTHESIS FOR WALLERIAN NERVE DEGENERATION. YAWO, H. and KUNO, M. Dept. Physiol., Kyoto Univ. Fac. Med., Sakyo-ku, Kyoto 606.

In the calcium hypothesis, it is assumed that Wallerian degeneration is induced by excessive Ca^{++} entering at the cut end of a nerve fiber and that the degeneration spreads progressively along the fiber from its cut end. This hypothesis was examined by intracellular recording from the cockroach giant axon, using the loss of excitability as a criterion for degeneration. When the axon was transected about 1 mm from the recording site, the axon showed a sudden depolarization and a drastic decrease in the input resistance. These two changes were however restored simultaneously within 30 min. About 22 hr after the transection, intracellular recording from the axon 150 μm from the cut end showed normal resting and action potentials. The conduction failure in degenerating axons was observed 60-80 hr after the transection in the majority of the axons examined. The initial conduction block in degenerating axons occurred at discrete patches at a random fashion, independent of the distance from the cut end. Thus, there was no evidence that Wallerian degeneration is initiated at the cut end of a nerve fiber and proceeds progressively along the fiber from its cut end. It is concluded that the calcium hypothesis is incompatible with the experimental observations.

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STRUCTURE THEORY AND EXCITABLE SYSTEM OF ELECTRICAL ACTIVITY OF NERVE. MATUMOTO, M. and SANO, M. Dept. of Physiol., Sch. of Dent., Showa Univ., Tokyo 142

One of the authors (Matumoto) lately presented a structure theory for electrical activity of nerve fibers based on the equivalent circuit of a nerve fiber in its resting and excited states. The equivalent circuit of a nerve fiber is a double polarized layer. The inner layer is self polarized, and the outer layer (surrounding tissue of fiber) is polarized by discharge of current from the inner layer. In the resting state, resistance of the inner layer (Rm) has a certain high value which is reduced transiently to R_{ℓ} ($\fallingdotseq 0$) by a stimulating current. The resting equivalent circuit then changes to an excited state, so that excitation occurs and recovers to produce an action potential. Rm and the other parts of the equivalent circuit in the resting state are called the active element and field of excitation, respectively. Together they constitute the "excitable system". This structure theory permits the making of a hardware model, Matumoto's Model of Excitable System (MMES), with electric elements which can be tested under various experimental conditions. Results of experiments obtained under known conditions were the same as those for a nerve, and under conditions not yet reported several new facts were obtained which were later proved for nerves.

The present report discusses electrical behavior obtained by theoretical treatment of MMES which might demonstrate the appropriateness of the structure theory.

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TRANSMEMBRANE MACROMOLECULE ROTATION MODEL — MOLECULAR HYPOTHESIS OF MEMBRANE EXCITATION. GOTO, K. Dept. of Pathology, Tohoku University School of Medicine, Sendai 980

A hypothesis concerning the molecular changes in the nerve cell membrane during excitation in presented. Based upon established principles about the interaction of hydrophobic and hydrophilic ligands and upon recent facts concerning the contraction of neurofilaments, the hypothesis accounts for the initiation of the action potential, its conduction along the nerve axon without attenuation, and chemical transmission at the presynaptic membrane. When acetylcholine combines with the acetylcholine receptor (AChR) α subunit, hydrophobic subsites are exposed at the bound surface of AChR. Since hydrophobic portions are quite unstable, the subsites move to the center of the membrane, which is hydrophobic. It is postulated that the rotation of receptor subunits makes the center an ion channel. Ca^{++} flowing in through the ion channel causes the underlying filaments to contract and this contraction results in the conduction of an action potential along the axon. The contraction of the underlying filaments induces transmembrane rotation of globular proteins connected to the filaments. Igakunoayumi, 121, 201-203, 1982. Igakunoayumi, 122, 971-973, 1982. Tohoku J. Exp. Med. 139, 159-164, 1983.

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INFLUENCE FOR THE RESISTANCE AND CAPACITANCE OF THE LIVING BODY BY THE FINITE ALTERNATING POTENTIAL AMPLITUDE. SAITO, T. Dept. of Physiolog. Nippon Dental College Tokyo.

The resistance and capacitance have been measured by direct or alternating currents for declearing the physiological mechanism of the living body. One methode of them is the alternating bridge, but it showes only the rest condition. The finite alternating potential is invented to measure them in the transient and rest condition.

The applying potential is $E_m \sin\omega t$ and the electrical model of the living body is the series circuit of resistance r and capacitance c. The passing potential through it is v and the input resistance of the measuring apparatus is R. And τ is the deviation time of the applying and psassing potential and $t=n\pi-\tau$. When t is $n\pi$, v is $v_{n\pi}$, and ϕ is $1/\omega(R+r)c$.

$$\mp\left(\cos\omega\tau - \frac{\sin\omega\tau}{\tan\phi}\right) = \varepsilon^{-(n\pi-\tau)\omega\tan\phi}, \quad \mp v_{n\pi} = \frac{RE_m}{R+r}\sin\phi\cos\phi\left(\mp 1 - \varepsilon^{-n\pi\tan\phi}\right)$$

When $\omega=5.95 \times 10^3$(rad/s) and E_m is 0.502(V), r=16.8(MΩ), c=4.04(PF) and when $\omega=5.99 \times 10^3$(rad/s) and E_m is 11.1(V), r= 16.8(MΩ), c=2.81(PF) in the bullfrog sartorius muscle through microelectrode.

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CHANGE IN OPTICAL ACTIVITY OF STAINED NERVE. Watanabe, A. Dept. of Cell Physiology, Nat. Inst. Physiol. Sci., Okazaki 444

The optical activity of nerve, stained with certain dyes, changes when the nerve is excited. Nerves were taken from walking legs of the spiny lobster, cleaned, stained with dye solutions for 10-30 minutes and mounted on a chamber for the optical experiment. A lens, an interference filter and a polarizer were inserted between the light source and the preparation. An analyzer and an optical detector were placed in the optical path after the nerve. The output of the detector was amplified and fed to a signal averager. Experiments were performed under two particular settings of the azumuths of the polarizer and analyzer. In setting A the transmission axis of the analyzer was set 45° more clockwise than that of the polarizer. In setting B the azimuths of the polarizer and the analyzer were exchanged. In either setting one of the azimuthal angles was kept either in parallel with or perpendicular to the longitudinal axis of tne nerve. When the nerve was stimulated the intensity of the transmitted light changed transiently. The size of the intensity change was different according to the setting of the analyzer and the polarizer. The difference was considered as due to the change in nerve optical activity. The largest signal was obtained from a nerve stained with Merbromin (marcuridibromofluorescein), and the change was about 10^{-3} degrees (levorotation).

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THE GABA-INDUCED CURRENT FLUCTUATIONS AND THEIR ENHANCEMENT BY BARBITURATES. HIGASHI, H., HIJIRIKAWA, K.,*KUWAHARA, H. and NISHI, S. Dept. Physiol. and*Central Computer Lab., Kurume Univ. Sch. Med., Kurume.

A voltage-clamp analysis of the GABA-produced membrane currents in neurones of the isolated spinal ganglion of the cat has been carried out to study the gating kinetics of the GABA operated chloride channels. The power spectra of GABA current fluctuations followed single Lorentzian curves, and the corner frequency of these curves indicated the mean life time (τ) of the activated chloride channels to be about 6.8 ms at 36°C. The conductance (γ) of a single chloride channel was estimated from the power spectra or the variance of current fluctuations to be about 21 pS. When the chloride channels were activated by muscimol, a potent agonist of GABA, γ remained the same as they were activated by GABA, but τ prolonged to as much as 15.7 ms. Following inhibition of the uptake of GABA by Schwann cells using a Na-free Krebs or nipecotic acid (1 mM), the τ of GABA activated chloride channels prolonged to 9.5 ms, while that of muscimol activated channels remained unchanged. Pentobarbital (10-100 μM) dose-dependently prolonged τ, whereas it did not appreciably alter γ. At the concentration of 1 mM pentobarbital itself opened chloride channels which showed τ of 10 ms and γ of 18 pS. Thus the augmentation of GABA depolarization by barbiturates can be explained in terms of prolongation of the life time of activated chloride channels.

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EFFECTS OF DIHYDROPYRIDINE DERIVATIVES ON THE NEURONAL PROPERTIES.
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The 1,4-dihydropyridine derivatives, known as potent blockers for the voltage-dependent Ca^{2+} channels in heart, smooth and skeletal muscle cells, were applied to the neural preparations including rat pheochromocytoma clone PC12 cells, rat brain synaptosomes and slices.

In PC12 cells, ^{3}H-norepinephrine and ATP releases and ^{45}Ca uptake induced by high K^{+} depolarizations were inhibited by the derivative (nicardipine) with half inhibition doses of 10^{-8} to 10^{-7} M. However, the electrophysiological examinations revealed that the depolarization-triggered Ca^{2+} spikes and Ca^{2+}-dependent after-spike hyperpolarizations were uninfluenced by the treatment of 10^{-6} M nicardipine. Thus, it is suggested that PC12 cells have two voltage-dependent Ca^{2+} influx pathways; dihydropyridine-insensitive Ca^{2+}-spike channels and dihydropyridine-sensitive slowly inactivating channels.

In rat cerebral cortex synaptosomes, neither ^{3}H-GABA and ATP releases nor ^{45}Ca influxes were suppressed by 10^{-6} M nicardipine. In rat cerebral cortex slices, the GABA-release was not blocked by nicardipine. Thus care must be taken in concluding that the specific dihydropyridine binding reported in brain membrane fractions would be derived from the neuronal Ca^{2+} channels.

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STUDIES ON THE EXCITATORY AMINO ACID RECEPTORS OF THE INSECT NEUROMUSCULAR JUNCTION.
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The excitatory neuromuscular junction of insect (Tenebrio Molitor) was investigated using specific blocker (JSTX) and agonists of glutamate receptor. JSTX purified from Nephyla Clavata venom blocked the excitatory postsynaptic potentials (EPSPS) in the abdominal muscle of adult Tenebrio and larva (meal worm). Larval muscle, however, was found much less sensitive than that of adult. L-glutamate caused a large depolarization on the postsynaptic membrane in larval muscle but it produced a hyperpolarization in adult muscle. Iontophoretically applied glutamate produced both depolarizing (D type) and hyperpolarizing (H type) responses in adult muscle. Quisqualic acid act similarly to glutamate. Kainic acid suppressed EPSPS by blocking the nerve terminal spike in both larva and adult muscles. The results suggest a developmental change in glutamate receptors in insect which is responsible for different response to L-glutamate or sensitivity to JSTX.

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MUSCARINIC AGONISTS DEPRESS CALCIUM DEPENDENT POTASSIUM CONDUCTANCE OF MYENTERIC NEURONES.
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Intracellular recordings were made from AH (type 2) myenteric neurones of guinea-pig ileum. Muscarinic agonists, acetylcholine (ACh) and oxotremorine, decreased membrane potassium conductance (g_K). Calcium carried into the neurone by spike(s) increased g_K (g_{KCa}). The time course of the muscarinic change in g_K was compared to that of the changes in g_{KCa} following spike(s). The time course of conductance decrease were similar in both cases and had the same temperature coefficient. Concentration of muscarinic agonists (30-100 nM) which were too low to cause detectable decrease in g_K shortened the duration of g_{KCa} increase following spike(s). This was not due to reduction of calcium entry during spike(s). The conductance changes during muscarinic action and afterhyperpolarization did not add linearly. Low concentration barium (10-100 μM) mimicked muscarinic action of ACh. It is proposed that muscarinic agonists may reduce the availability of calcium at a site within the membrane which controls g_K.

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DEVELOPMENTS OF MEMBRANE EXCITABILITY AND INTRAMEMBRANOUS PARTICLES IN NEUROBLASTOMA × GLIOMA HYBRID CELLS. FURUYA, K., FURUYA, S.* and YAMAGISHI, S. National Inst. Physiol. Sci., Okazaki 444

The differentiation process of NG108-15 cells was analyzed using electrophysiological and freeze-fracture techniques. The maximum rate of rise of Na and Ca spikes were measured at every culture days after dBcAMP treatment which induced neural differentiation. The Ca spike began to appear at 2nd day of culture and complited at 5th day. Na spike began to appear late at 4th day and complited at 7th day. After then, these spikes remained constant during the longer culture ages and did not decrease even at 18th day. The distribution of intramembranous particles (IMPs) was rondom and their density increased in both P-face and E-face of the plasmalemma as the membrane excitability developed. Undifferenti ated cells in growth medium had 1400 IMPs/μm^2 in the P-face and 280/μm^2 in the E-face at 2-6 days culture. After the cells were differentiated with dBcAMP for 1 week, the density of IMPs was 2600/μm^2 in the P-face and 540/μm^2 in the E-face. The analysis of size distribution showed the increase of the number of medium (7.5-10 nm) and large (>10 nm) particles.

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ELECTROPHYSIOLOGICAL PROPERTIES OF CULTURED CEREBELLAR NEURONS EXPLANTED FROM CHICK EMBRYO. MORI-OKAMOTO,J., ASHIDA,H. and TATSUNO,J. Department of Physiology, National Defense Medical College, 3 Namiki, Tokorozawa, Saitama 359.

Action potentials were intracellularly recorded from cultured cerebellar neurons of 25-40 days old. Most neurons showed spontaneous discharges of repetitive single spikes or bursts with or without single spikes, which were inhibited by Mn^{2+}. IPSP-like potentials with reversal potentials of -50--55mV were also observed in some neurons. Action potentials were evoked by a depolarizing pulse at resting membrane potentials in all neurons tested. These evoked spikes were not completely blocked by 10^{-5}g/ml tetrodotoxin(TTX). Such TTX-resistant component was more frequently seen when tetraethylammonium and/or 12mM Ca^{2+} were added to the medium. Action potentials could be recorded also in Na^+-free solution. This Na^+-independent component was blocked by Co^{2+}. These results suggest that Na and Ca ions are combinedly involved in spike generating mechanisms in cultured chick cerebellar neurons, and also that they may be related to the maturation process of excitable somatic membranes.

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EFFECT OF SERUM-FREE MEDIUM ON DEVELOPMENT OF EXCITABILITY IN A CLONAL MOUSE MYOGENIC CELL LINE. AMAGAI, Y., IIJIMA, M. and KASAI, S. Dept. of Physiol., Tohoku Dent. Univ., Koriyama-shi, Fukushima 963

Cells of a mouse myoblast line MC3T3-A1 clone M13 were cultured either in serum-supplemented medium (S^+M; Ham's F-12 + 10% newborn bovine serum) or in serum-free medium (S^-M; "MM-1", Florini & Roberts, 1979). The cultures were then compared if myotubes differentiated in S^-M could express a series of development of excitability as those in S^+M, which generate Ca spikes at the early stage and Na spikes when they mature. After M13 cells had achieved confluence in S^-M, they formed myotubes as in S^+M and developed Ca action potential-generating mechanism. However, they could not generate Na spikes, indicating that the development of excitability in these myotubes was arrested before the maturation. This failure of S^-M in cultivating M13 cells sufficiently could be restored in part by addition of transferrin at doses up to 0.1 μM so that Na spikes having a maximum rate of rise of 30±3 V/sec which is about 1/10 of the control could be elicited. These results suggest that the development of Na spike-generating system in M13 myotubes depends partially on some transferrin-associated metabolism during the differentiation.

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SELECTIVE REDUCTION OF Ca SPIKES IN THE NERVE CELL CULTURED IN A SERUM-FREE MEDIUM. YAMAGUCHI, K. Dept. of Physiol., Fac.of Med., Univ. of Tokyo

Serum-free culture of nerve cells was undertaken in order to study effects of serum factors on physiological properties of the nerve cell membrane. Neurons of the dorsal root ganglia from the adult guinea pig were cultured in a serum-free medium, which contained DMEM(½volume), Ham's F12 (½volume), insulin(5μg/ml), transferrin(30μg/ml), putrescine(100μM), progesterone(100μM) and selenite(30nM). Nerve cells in the serum-free medium grew well-developed neurites and survived for more than 3 weeks. Intracellular recording with a glass microelectrode revealed that the nerve cells grown in the serum-free medium exhibited essentially the same resting membrane properties(resting potential, resistance and capacitance) as those of nerve cells grown in the serum-supplemented medium. Although the nerve cells grown in the serum-free medium generated unchanged Na spikes, their capability to generate Ca spikes was significantly reduced during growth in the serum-free medium. Namely, the maximum rate of rise of the Ca spike was approximately half of that of control nerve cells on day 5. This observation indicates that unknown substances in the serum may regulate development of Ca channels in the nerve cell membrane.

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MORPHOLOGICAL AND ELECTRICAL MATURATION OF MOUSE NEUROBLASTOMA CELLS IN CULTURE. TSURUOKA, M., MATSUI, Y. and MATSUI, A. Dept. of Physiology, School of Dentistry, Showa University, Shinagawa-ku, Tokyo 142

Dibutyryl cyclic AMP, aminopterin, or serum-free culture medium induce neurite formation and the maturation of neural properties of mouse neuroblastoma cell. However, the question of a possible link between the processes of neurite formation and electrical maturation remained unsettled. In the culture media containing 1mM db-cAMP, 10^{-5}M aminopterin and serum-free, about 50% of the cells extended neurite after 1-5days, while only 10-20% of the cells generated active response to electrical stimulation. Further incubation led to a increase in neurite length and the proportion of excitable cells, but the proportion of cells with neurite remained constant.

Three types of responses to electrical depolarizing stimuli were passive, delayed rectification and active response. The resting potential of the maturated cells,which generated active responses, increased to a deeper level than that of cells exhibited delayed rectification and passive response. The effective membrane resistances of the maturated cells were higher than that of the other cells in spite of long neurites and relatively large cell soma. These results indicated that morphological differentiation is followed by electrical maturation and electrical membrane properties are expressed in the following sequence: passive, delayed rectification and active response.

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OPTICAL DEMONSTRATION OF MEMBRANE POTENTIAL RESPONESE IN EARLY EMBRYONIC SEMILUNAR GANGLION. SAKAI, T., FUJII, S., HIROTA, A. and KAMINO, K. Dept. of Physiology,Tokyo Medical and Dental University School of Medicine, Bunkyo-ku, Tokyo 113

Changes in absorbance of voltage-sensitive merocyanine-rhodanine dyes were used to monitor electrical responses in the semilunar ganglion of 4-10-day-old chick embryos. Stimulation of the afferent nerve fibers with a suction electrode led to changes in optical absorption of neurons of the ganglion. These changes depended critically on the wave-length of the incident light, and were eliminated at a wave-length of 620nm where the voltage dependent absorption change of the dye disappears. In the great majority of the 4-10-day-old embryonic ganglia, the optical membrane potential responses varied with the intensity of the stimulus and had the nature of an electrotonic spread. This non-conducted optical response could be classified into two types, according to shape: Type I was symmetric and resembled the optical responses elicited with depolarizing and hyperpolarizing square current pulses and Type II exhibited a "local-response"- like initial upstroke followed by the steady-state plateau evoked by larger depolarizing pulses. In some ganglia at the 6-10-day-old developmental stages, conducted optical responses were detected, suggesting that action potential activity of the semilunar ganglion cells is initiated during the development of the 6-10-day-old embryos.

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DEVELOPMENT OF IONIC CHANNELS IN THE CLEAVAGE-ARRESTED, DIFFERENTIATING EMBRYO OF AN ASCIDIAN. HIRANO, T. and TAKAHASHI, K. Dept. of Neurobiology, Inst. of Brain Research, School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113

We previously reported that the ascidian embryo whose cleavage was arrested with cytochalasin B just after the fertilization differentiated to a cell of epidermal type. Changes of ionic channels during development in the cleavage-arrested 1-cell embryo have been studied. The development was divided into 4 stages, S1, S2, S3 and S4. At S1, which was from the fertilization to the late gastrula of the control embryo, Na and egg Ca channels decreased. At S2, which was to late neurula, Na and anomalous rectifier channels and total membrane capacitance increased. At S3, which was to tail bud stage, Na and anomalous rectifier channels and the membrane capacitance decreased. At S4 differentiated Ca and anomalous rectifier channels increased and Ca-induced K current appeared. Actinomycin D, an inhibitor of transcription, when applied soon after the fertilization, inhibit the increase of ionic channels at S4 without affecting the changes from S1 to S3. The results indicate that the changes in various ionic channels occurred somewhat synchronously during development, and suggest that the changes from S1 to S3 are probably maternally regulated but that the changes at S4 need new transcription.

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EFFECTS OF PERGOLIDE AND ITS DERIVATIVES ON THE MOLLUSCAN GIANT NEURONES. KU, B. S. and TAKEUCHI, H. Dept. Physiol., Gifu Univ., Sch. Med., Tsukasa-machi 40, Gifu 500

On the two dopamine (DA)-sensitive giant neurones, PON (periodically oscillating neurone, excited by DA) and TAN (tonically autoactive neurone, inhibited by DA), identified in the suboesophageal ganglia of an African giant snail (Achatina fulica Férussac), effects of synthetic ergot derivatives, including pergolide and lisuride which are considered to be DA agonists, were examined.

Of the substances examined, three of the ergot derivatives related to pergolide, D-8,9-didehydro-6-propylergoline-8-methanol, D-6-methyl-8β-(2-(methylsulfinyl)ethyl)ergoline and D-2-chloro-6-methyl-8β-(2-(methylsulfinyl)ethyl)ergoline, showed excitatory effects on PON, while pergolide (D-8β-((methylthio)methyl)-6-propylergoline) and lisuride (N-D-6-methyl-8-isoergolenyl-N',N'-diethylcarbamide) had no effect.

On the other hand, only D-6-methyl-8β-(2-(methylsulfinyl)ethyl) ergoline had any excitatory effects on TAN.

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EFFECTS OF AROMATIC AMINO ACID DERIVATIVES ON THE MOLLUSCAN GIANT NEURONES. III. TAKEUCHI, H. and *ARIYOSHI, Y. Dept. Physiol., Gifu Univ., Sch. Med., Tsukasa-machi 40, Gifu 500; *Central Lab., Ajinomoto Co., Inc., Suzuki-cho, Kawasaki-ku, Kawasaki 210

The marked inhibitory effects of N-β-phenylpropionyl-L-tyrosine (the critical concentration (c. c.), 3×10^{-7}--10^{-6}M) and N-β-phenylpropionyl-L-tryptophan (c. c., 10^{-6}M) on an identifiable giant neurone, TAN (tonically autoactive neurone), of Achatina fulica Férussac have been previously reported. In the present study, effects of their newly synthesized derivatives were compared.

N-β-(3-Chlorophenyl)propionyl-L-tyrosine and N-β-(3-chlorophenyl)propionyl-L-tryptophan, having a chlorine in the phenyl group, showed inhibitory effects (c. c., 3×10^{-5}M) weaker than those of the two original compounds.

Effects of N-phenylpropargyloyl-L-tyrosine (c. c., 3×10^{-6}M) and N-phenylpropargyloyl-L-tryptophan (c. c., 10^{-6}M), having a triple bond in the carbon chain attached to the phenyl group, were similar to or slightly weaker than those of the original compounds.

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ELECTROPHYSIOLOGICAL IDENTIFICATION OF THE EXCITABLE MEMBRANE IN THE MYELINATED NERVE FIBERS OF Penaeus japonicus. HSU, K.* Dept. of Cell Physiology, Nat. Inst. Physiol. Sci., Okazaki 444

Histological studies on the nerve fibers of the shrimp show that the axon and the myelin sheath are separated by an unique microtubular sheath and a large submyelinic space. Action potentials similar to those recorded intracellularly can be observed by glass pipette electrode placed in the submyelinic space. Many attempts to measure the resting potential of the axon have been failed (Hsu et al. KEXUE TONGBAO 20: 380-386, 1974). These have obscured the location of the excitable membrane in nerve fibers of the shrimp. A microelectrode containing Lucifer Yellow CH was inserted into the axon through the synaptic region of the medial giant fiber. Resting potentials of about -60 mV and action potentials of 50 - 70 mV could be observed only when the dye distributed within the axon. Frequently, the same procedure resulted in staining of not only the axon but also the submyelinic space. In these cases, action potentials of various sizes could be observed, but the resting potential could never be measured. From this and other experiments of putting ligatures on the medial giant fiber, the excitable parts of the axon membrane were identified to be localized in the synaptic regions.

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A STUDY ON ORGANIC ACID SALTS-INDUCED ENHANCEMENT OF LOCAL ANESTHETICS EFFECT: INTRACELLULAR RECORDING OF CRAYFISH GIANT AXON. YAMASAKI, J.*, MIYOSHI, M., IMOTO, T. and HIJI, Y. Dept. of Physiology, Tottori Univ. School of Medicine, Yonago 683

Intracellular recording were performed on crayfish giant axon. When the axon was treated by procaine together with sodium salicylate, action potential was depressed in the amplitude more strongly than that of procaine alone and recovered to the control size with the long time after removing the drugs. Although procaine or salicylate itself hardly affected resting potential, the mixture depolarized it remarkably. The maximum rate of fall of action potential was significantly suppressed without affecting rate of raise by salicylate alone, suggesting that the potassium ion permeability was preferentially decreased by salicylate treatment.

On the other hand, sodium propionate also showed similar enhancing effect, but no significant change on resting potential. Propionate itself did not affect action potential. Thus, the enhancing mechanism of propionate seems to differ from that of salicylate.

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EFFECTS OF TEMPERATURE ON THE HYPERPOLARIZING RESPONSE IN CRAYFISH NEURON SOMA. ARITA, A. and HAYASHI, H. Dept. of Physiol., Saitama Med. Sch., Moroyama, Iruma-gun, Saitama 350-04

Hyperpolarizing responses (HR) of the somata of median giant fibers of crayfish, Procambarus clarkii, were investigated under current clamp and voltage clamp in normal saline at the temperature of 20 and 5 °C. The responses were classified into two types: N-type which showed the negative slope region of the voltage clamp I-V curve and G-type. There were no significant differences in resting electrical constants and HR parameters between N-type and G-type responses. On decreasing the temperature from 20 °C to 5 °C, resting potential decreased by 24 %, membrane resistance increased by 30 %, no change in the peak amplitude of HR, plateau amplitude increased by 38 %, slow EMF shift at the plateau decreased by 3.3 mV, and fast one increased by 7.8 mV. Voltage dependent conductance in N-type I-V curve decreased, and voltage independent conductance in G-type I-V curve also decreased at low temperature.

The nonlinearity of instantaneous I-V curve in peak phase was observed at 5 °C as well as at 20 °C.

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THE EFFECT OF ORGANIC ACIDS AND NUCLEOTIDES ON THE ACTION OF ANESTHETICS AS STUDIED IN THE RAT SCIATIC NERVE IN VIVO. MINAMI, Y.* and HIJI, Y. Dept. of Physiology, Tottori University School of Medicine, Yonago 683

The present studies were performed by behavioral observation of foot drop caused by the sciatic nerve block. By addition of 0.7% Na--salicylate to 0.5% procaine, the analgesic duration was prolonged to twice as that of procaine alone. Similar enhancing effect was observed in cases of formate and acetate. Contrarily, fatty acids having more than three carbon atoms such as propionate and isovalerate showed suppressing anesthetic effect. ATP and AMP also shortened the duration.

The conduction block of the peroneus nerve action potential evoked by stimulation of the sciatic nerve was also electrophysiologically recorded in vivo. Salicylate injected near sciatic nerve in the groin together with procaine prolonged the recovery time as twice as that for procaine alone. Whereas, both the onsets of block and recovery in the nerve activity were significantly shortened in the presence of propionate. The electrophysiological data seem to explain the behavioral observation.

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SLOW RHYTHMIC MEMBRANE POTENTIAL CHANGES IN HAMSTER SUBMANDIBULAR GANGLION CELLS. SUZUKI, T. Dept. of Oral Physiology, Matsumoto Dental College, Shiojiri, Nagano 399-07

A small number of hamster submandibular ganglion cells generated slow rhythmic membrane potential changes (SRMPCs), which were considered to be pacemaker activity. The amplitude of the SRMPC depended on the resting E_m. The most positive and negative E_m levels of SRMPC were -58.0 ± 3.3 mV and -87.6 ± 2.8 mV (mean ± S.D.), respectively. The latter E_m level corresponded to E_K of this ganglion cell. The interval between the cycles of SRMPC was 3.7 ± 2.9 minutes (mean ± S.D.). This interval did not depend upon the resting E_m. In eserine-treated preparations, the SRMPC was triggered by preganglionic or sometimes postganglionic repetitive stimulations. Bath-applied carbachol or DMPP were also effective. Using these effects, more than 30% of those cells which did not exhibit spontaneous activity also generated damped SRMPCs. The ionic basis of the SRMPC characterized in this way was analyzed electrophysiologically and pharmacologically. From the results, it was concluded that the SRMPC is generated by slow periodic activation of $G_{K(Ca)}$ brought by changes in $[Ca^{2+}]_i$. In addition, the activation of $G_{Ca(V)}$ for Ca^{2+} entry from external medium may occur during the rising phase near the peak of SRMPC. Mitochondria might be responsible for the generation of SRMPC as internal regulatory organs of Ca^{2+}.

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MEMBRANE PROPERTIES OF RETINAL HORIZONTAL CELLS IN CULTURE. TACHIBANA, M. Dept. of Neurobiology, Harvard Medical School, Boston 02115 U.S.A. (Present address: Dept. of Information Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki, 444)

The membrane properties of solitary horizontal cells, dissociated from papain-treated goldfish retinas and maintained in vitro, were analyzed under current- and voltage-clamp conditions. The resting membrane potential was mainly determined by K-permeability. The cells produced all-or-none action potentials. Their current-voltage relationships were non-linear, with inward (anomalous) and outward rectifications. Voltage-dependent ionic currents consisted of: 1) a Ca current which was activated by depolarization beyond -45 mV, blocked by 2 mM Co ions, and inactivated by accumulations of intracellular Ca ions, 2) a K current through the anomalous rectifier which was activated by hyperpolarization below the resting potential and blocked by 10 mM Cs or 1 mM Ba ions, 3) a transient outward current which was activated by depolarization beyond -25 mV, inactivated with an exponential time course (time constant; about 500 msec at +20 mV), and suppressed by holding the membrane potential beyond -30 mV or by 10 mM 4-AP, and 4) a maintained outward current which was activated by depolarization beyond -20 mV and blocked by 20 mM TEA. A Ca-mediated K current was not detected.

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ACTION POTENTIALS DEPENDENT ON MONOVALENT CATIONS IN MOUSE OVARIAN OOCYTES.

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Ovarian oocytes were isolated from the adult mouse and intracellular recording was performed. The resting potential was approximately -7 mV in standard solution, and the oocyte showed regenerative responses.

Action potentials dependent on divalent cations (Ca^{2+},Sr^{2+},Ba^{2+},Mn^{2+}) were observed under Na^+-free conditions. Such spikes were insensitive to tetrodotoxin (TTX) and were suppressed by Ca blockers, indicating the existence of Ca channels in the ovarian oocyte membrane.

Action potentials were also detected in Ca^{2+}-free solution containing 2 mM EGTA. The overshoot of the spike showed a slope of 39 mV for a tenfold increase in $[Na^+]_o$, and a saturation was found when $[Na^+]_o$ was highly elevated. The spike was resistant to TTX and was blocked by polyvalent cations such as Co^{2+}, Cd^{2+}, Mn^{2+} and La^{3+}. Li^+ substituted for Na^+ in generating spikes, while Rb^+ did not. The overshoot and maximum rate of rise of the Na spike became smaller when Ca^{2+} was present in the bathing solution, suggesting a competition between Na^+ and Ca^{2+}.

It is concluded that the ovarian oocyte membrane of the mouse has voltage-dependent Ca channels, and divalent and monovalent cations can pass through the channels to produce action potentials.

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INTRACELLULAR ANALYSIS OF MEMBRANE PROPERTIES OF FROG PRIMARY AFFERENT FIBERS.

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Intracelluar recording from primary afferent fibers (PAF) in frog dorsal root revealed distinct outward going rectification operating in the potential range more positive than - 100 mV. The longest time constant of charging and discharging phase of an electrotonic potential was often found to exceed 50 msec. A conventional Hodgkin-Huxley model of a node of Ranvier, proposed by Hille (1969), failed to mimick membrane behaviour of real fibers. On the other hand, a modified node model which incorporated recently found slow K channel (Dubois, 1981) successfully explained non-linear current-voltage relationship of PAF. Deactivation process of slow K conductance can partly contribute to slow charging phase of a hyperpolarizing electrotonic potential, but the observed electrotoinc potential showed far slower discharging phase than the predicted one. Other capacitative components such as capacitance of internodal membrane seem to be responsible for slow time course of a PAF electrotoinc potential.

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THE ROLE OF AFTERHYPERPOLARIZATION IN REPETITIVE FIRING OF APLYSIA BUCCAL GANGLION CELLS.

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In the nerve cell, the application of a constant depolarizing current across the membrane gives rise to repetitive discharges, whose frequency is dependent on the current strength. Aplysia buccal ganglion cells were classified into two groups owing to different ionic dependency of action potentials, (Na+Ca)-dependent cells and Na-dependent cells. Repetitive firing in such two groups of the neurons responding to the injected depolarizing current was investigated, respectively. In both groups, the slope of the frequency-current (f-I) plots showed a high gain in Ca-free artificial seawater (ASW) and a low gain in Ca-rich ASW in comparison with that in normal seawater (NSW). We have demonstrated that the peak amplitude of afterhyperpolarization (AHP) following the action potential was decreased in Ca-free ASW and increased in Ca-rich ASW. This result indicates that the frequency of the repetitive firing is regulated by the AHP amplitude depending on extracellular Ca concentration, and suggests that the Ca ions play an important role in the regulation of repetitive firing. Moreover, in the Na-dependent cells a spike was generated in Na-free ASW containing 4-aminopyridine.

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CRYSTALLOGRAPHIC CHANGE OF LYSOSOME-LIKE GRANULE STORED CALCIUM BY PENTYLENETETRAZOLE-INDUCED BURSTING ACTIVITY IN SNAIL NEURONS.

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To obtain a clue concerning the calcium release mechanism from lysosome like granules (LLG) during pentylenetetrazole(PTZ)-induced bursting activity in snail neurons a crystallographic examination was performed with a newly developed curved position sensitive proportional counter type X-ray microdiffractometer.

Calcium was stored in LLG at least in the forms of calcium hydrogen orthophosphate and calcium diphosphate in the normal state. After PTZ treatment of the ganglion, these crystallographic characters disappeared; and rinsing with normal snail Ringer solution restored the normal diffraction pattern. An amorphous state molecular change was observed by PTZ treatment.

These results suggest that in snail neurons, calcium is stored in LLG in the form of the above-mentioned two types of calcium compound and during bursting activity LLG loose their normal crystallographic pattern as well as their normal morphological structure.

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A DESCRIPTION OF ACTIVATION IN CALCIUM CHANNELS BASED ON TAIL AND TURN-ON MEASUREMENT. TSUDA,Y. BROWN,A.M.* and WILSON,D.* 1st Dept.Internal Med. Kyushu Univ. Fukuoka and Dept.Physiol.Biophys. Univ. Texas Galveston

Activation,or turn-on,was compared with deactivation,or turn-off(tail) Ca current. The experiments were done on isolated nerve cell body of Helix aspersa,voltage clamped and internally perfused using a combined suction-ionpipette-glassmicroelectrode method. Ca current was isolated by suppression of Na and K current. Tail current,produced by short voltage clamp pulses,were fit by sum of 2 exponentials; fast τs and slow τs, 0.2ms and 1.2ms,respectively.These two were both due to Ca channel closure. Third component was not always present,which does not appear to be related non specific current. In the cells without slowest component,turn-on was well described by an m^2 H-H activation process having 2 timeconstants τm and $\tau m/2$. However,taus of tail current, τf and τs,had variable ratio(6-8) rather than 2 required by m^2 model.The fact that there were 3 exponential in the systen response indicates a minimum of 4 state in a transition state model of activation.The effect of cooling to slow turn-on without affecting tail taus were explained by making the leading forward rate constant of the 4 statemodel much more temperature sensitive than the others.

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CALCIUM CURRENT INACTIVATION AND ACCELERATING ACTION OF ETHANOL ON THE INACTIVATION PROCESS. OYAMA, Y., NISHI, K. and AKAIKE, N.(*) Dept. of Pharmacology, Kumamoto University Medical School, Kumamoto 862 and (*) Dept. of Physiology, Fac. of Medicine, Kyushu University, Fukuoka, Japan.

Effects of ethanol on the Ca current were investigated in the snail neurones perfused intracellularly and voltage-clamped by a suction pipette technique. Ethanol decreased the peak amplitude of the current and produced the acceleration of the current inactivation in a dose-dependent manner at all membrane potentials. Ethanol did not affect the rising phase and non-specific outward current. Double-pulse experiments showed also the acceleration of the current inactivation in the presence of ethanol. Accelerating the decay phase of the current appears to be due to speeding up of normal inactivation mechanism rather than a time-dependent block of open channels by ethanol molecules. The modification in the Ca current inactivation may be resulted from conformational alternations in the lipid milieu surrounding the Ca channels by ethanol, since the alcohols are known to perturb the structure of lipid bilayer membrane. We can not rule out the possibility that ethanol affect the protein part of the Ca channel.

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Ca^{2+} CHANNEL ACTIVATING ACTION OF MAITOTOXIN IN CLOANL RAT PHEOCHROMOCYTOMA CELLS AND RAT BRAIN SYNAPTOSOMES. TAKAHASHI,M. AND OHIZUMI,Y.Lab. of Neurochemistry and Lab. of Pharmacology,Mitsubishi-Kasei Inst. of Life Sciences, Machida, Tokyo.

Effects of maitotoxin,the most potent marine toxin known,were studied using rat pheochromocytoma cells(PC12)and rat brain synaptosomes. In PC12 cells,maitotoxin induced a dose-dependent(3×10^{-9}-3×10^{-8}g/ml)increase in both $Ca^{2+}$and Na^{+} influxes. The maitotoxin-induced Ca^{2+} influxes into PC12 cells was observed even in the absence of external Na^{+}.But in the synaptosomes,maitotoxin induced Ca^{2+} influx without any effects on Na^{+} influx. In both the preparations,the maitotoxin-induced Ca^{2+} influx was markedly inhibited by Ca^{2+} channel blockers such as Mn^{2+},Co^{2+},verapamil and nicardipine and by a local anethetic,tetracaine.The maitotoxin-induced Na^{+} influx into PC12 cells was markedly inhibited by treatment with tetracaine, but was not affected by tetrodotoxin,veraapmil or nicardipine.These results suggest that maitotoxin directly activates Ca^{2+} channels of PC12 cells and rat brain synaptosomes.It is also suggested that in PC12 cells, maitotoxin increases Na^{+} permeability which occurred through tetrodotoxin-insensitive pathways.

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Effects of Ca-antagonist and antispasmodic drugs on the changes in membrane potential evoked by dopaminergic agonists in the rat submandibular gland cells. MURAKAMI, H. and SANO, M.* Dept. of Physiology, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271 and Dept. of Oral Physiology, Showa University School of Dentistry, Shinagawa-ku, Tokyo 142*.

We have already reported that the dopamine (DA) receptors are present in the rat submandibular gland cells. It is suggested that Ca play an important role in the electrical response mediated by muscarinic receptors and many others. In the present study, We reinvestigated the effects of DA receptor antagonists, sulpiride (Sul), butaclamol (Buta) and bulbocapnine (BULB) on the electrical responses evoked by DA and apomorphine (Apo) and in an attempt to study the role of Ca in the responses, effects of Ca-antagonist , verapamil (Ver) and antispasmodics, Aspaminol (Aspa), benactyzine (Bena) and papaverine (Pap) on the responses were investigated by the microelectrode method. Sul (10^{-6} M), Buta (10^{-6} M) and BULB (10^{-6} M) did not inhibit the responses. Bena (10^{-6} M) and Aspa (10^{-6} M) had no influence on the responses. Ver (10^{-6} M) and Pap (10^{-6} M) inhibited the responses. These findings suggested that the nature of DA receptors in the submandibular gland cells is somewhat different from that in the brain, and that the response evoked by DA seems to be associated with Ca from extracellular Ca source rather than with Ca from intracellular Ca store sites in the gland cells.

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FATTY ACIDS AND THE EXCITABILITY OF THE SQUID GIANT AXON. TAKENAKA,T., HORIE,H. and HORI,H. Department of Physiology, School of Medicine, Yokohama City University, Urafunecho,Minamiku,Yokohama 232

The effects of fatty acids on the excitability of the squid giant axon were studied by using a voltage clamp. When 2-decenoic acid was applied externally at the concentration of 1.5 mM in artificial sea water,it depolarized the membrane at a few millivolts and blocked the action potential. The membrane resistance was not changed by this drugs. The maximum value of the early inward-current was decreased and reached the steady state value at 15 minutes after application. This chemical dose not affect the late outward-current but only affects the early inward-current. These effects were completely reversible by washing with artificial sea water. Fatty acids less than 9 carbon atoms have no effect on either the early inward-current or late outward-current. The effects of fatty acids of the same chain length were compared. Sebacic acid ,which has carboxyl groups at both ends of the chain, has no effect on the early inward-current or the resting potential. These data show that the suppression of the sodium-current depends on the number of carbon atoms and the shape of fatty acids.

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MONOCLONAL ANTIBODIES AGAINST MUSCLE MEMBRANE PREPARATION FROM MUSCULAR DYSTROPHY CHICKENS. KURODA, Y. AND UONO, M.* Dept. of Neurochemistry, Tokyo Metropolitan Inst. for Neurosciences and Tokyo Metropolitan Neurological Hospital, Fuchu-shi, Tokyo 183.

In order to investigate the cause of Duchenne-type muscular dystrophy, inherited muscular dystrophy chickens have been used as a model animal. There are accumulating data which suggest that the disease might come from some defect(s) of cell membrane of myotubes. Plasma membrane fractions were prepared from pectoralis muscle of dystrophy(line 413) and normal(line 412) chickens just after their hatching. Spleen cells of the mice which had been immunized with the membrane preparations were fused with myeloma cells (NS-1). Resulting hybridoma were screened by enzyme-linked immunosorbent assay (ELISA) using plates in which two membrane preparations (dystrophy and normal) were separately fixed. Hybridoma supernatants from 3 wells of 384 appeared to have different responses to the dystrophy membrane and to the normal membrane. The result shows a possibility to get monoclonal antibodies against "dystrophy-specific" or "dystrophy-defficient" antigens by this method.

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SUPERNORMAL RESPONSE AND CONDUCTION IN DEPRESSED CANINE VENTRICULAR MUSCLE EXPOSED TO HIGH POTASSIUM. IINUMA, H., KATO, K.*, DREIFUS, LS.*, MICHELSON, EL.* The Cardiovascular Institute, Minato-Ku, Tokyo, and Lankenau Hospital, Philadephia, Pensilvania.

The size and conductivity of the premature response were compared with those of basic ones in canine ventricular tissue perfused with a high K^+(21mM) solution. After 8 driving stimuli(S1) of 0.5Hz, premature stimuli(S2) were applied at variable intervals. Action potentials(AP) elicited by S1 and S2 of equal strength and duration were recorded at proximal(P) and distal(D) points 3-6mm apart. The amplitude, duration of AP and interelectrode conduction time were examined. The basic response recorded at P was small (30±6mV, 34±12msec)(mean±SD) and did not conduct to D. However, a premature response elicited between 188±58 and 523±107msec after S1 was larger than the basic one and did conduct successfully to D, althouth with delay. Its amplitude, duration and conduction velocity decreased progressively as the S1-S2 interval was increased. Verapamil(5×10^{-6} g/ml) depressed and isoproterenol(10^{-7}g/ml) augmented both basic and premature responses; augmented premature responses were still observed in the presence of propranolol(10^{-4} g/ml). There was no evidence for either summation or oscillatory potentials as the mechanism. Additional experiments using locally depressed tissue sandwiched between normal tissues revealed that spontaneous repetitive responses were elicited following the augmented premature depolarization.

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INWARD RECTIFIER K CHANNEL IN THE RABBIT VENTRICULAR CELLS. KAMEYAMA, M., KIYOSUE, T., SOEJIMA, M., NOMA, A. and IRISAWA, H. Ntnl. Inst. Physiol. Sci., Myodaiji, Okazaki 444. Dept. Physiol., Med. Coll. Oita, Oita 879-56. Dept. Int. Med., Jikei Univ. Sch. Med., Tokyo 105.

A single-channel current was recorded from cell-attached membrane patches of single rabbit ventricular cells with fire-polished glass pipettes filled with 150 mM KCl in the normal Tyrode solution. The slope conductace at the resting potential ($\approx$ -82mV) was 47 $\pm$ 7 pS (mean $\pm$ S.D., n=16). This channel was identified as the inward rectifier channel since; 1) high density of the channel (2-3 channels/patch), 2) strong inward rectification in the I-V curve of the single-channel current, 3) dependency of the single-channel conductance on the external K^+ concentration (20-150 mM), and 4) sensitivities to Cs^+ and Ba^{2+} (0.01-1 mM). Open and closed time histograms of the channel showed single and two exponential components, respectively. The time constant of each component was voltage dependent, indicating that the macroscopic current through the inward rectifeir channel is time- and voltage-dependent.

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SINGLE CHANNEL ANALYSIS OF THE OUTWARD K CURRENT IN RABBIT A-V NODE CELLS.
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The patch clamp method was applied to single A-V node cells isolated from rabbit heart using collagenase. When the electrode contained 5.4 mM K, depolarizations of the cell-attached membrane from the resting potential induced outward single channel currents. The reversal potentials of the current at the K concentration of 5.4, 20 and 130 mM in the electrode agreed with those given by the Nernst equation, indicating that this channel is selective for K ions. The slope conductance near the reversal potential was 15 pS in 5.4 mM K at the outer side of the membrane. The conductance decreased at +80 mV depolarization from the resting potential, and thus the channel showed inward-going rectification. Depolarization increased the probability of the channel opening from 0.1 at -40 mV to 0.5 at +20 mV. In the inside-out patch, the kinetics and the conductance of this channel were not affected by raising Ca concentration from pCa 8 up to 5.3 in the bath solution. The channel was selectively blocked by ATP(>1mM) applied to the inside of the membrane, but not with ADP.

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Single Na channel current in cultured cardiac muscle cells of chick embryo.
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The gigaohm-seal patch clamp technique (outside-out: Hamill et al., 1981) was applied to record single Na channel current in cardiac muscle. Ventricular cells from 10-11 days-old chick embryo were dispersed with trypsin and maintained on glass cover slips in culture for 2 to 4 days. Inward directed single channel current was evoked with a depolarizing clamp pulse to -20 -40mV from holding potentials of -70 -80mV. Single channel conductance calculated from currents obtained at -40 and -20mV was 8.5pS at 13-14°C. Poisson analysis of the frequency of open times gives single exponential distribution with a mean channel closing rate of 605/sec, suggesting that only one kind of channel is functioning under this condition. Exposure to 3-100nM tetrodotoxin(TTX) did not significantly alter either single channel conductance, closing rate or mean open time, however, depressed the frequency of opening dose-dependently (K_D=22nM). The opening frequency of channels with short latencies was more sensitive to TTX than that of channels with long opening latencies, suggesting the existance of two pharmacologically separable types of Na channels.

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PROPERTIES OF Ca^{2+}-ACTIVATED K^+ CHANNEL IN CULTURED FIBROBLASTS: POSSIBLE ROLE OF CALMODULIN.
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The oscillation of membrane potential is caused by the recurrent stimulation of Ca^{2+}-activated K^+ channels in mouse fibroblastic L cells. These repeated hyperpolarizations were blocked by quinine, quinidine and TEA, but were not affected by 4-AP and cesium ions. The study with Ca^{2+}-selective microelectrodes demonstrated that the intracellular sub-micromolar Ca^{2+} ions stimulate the K^+ channels during the potential oscillation. Intracellular injection of Ca^{2+}, Sr^{2+}, Mn^{2+} or Ni^{2+} ions activated the K^+ conductance, but that of Mg^{2+} or Ba^{2+} did not. Intracellular injection of purified calmodulin also induced hyperpolarization. Putative calmodulin antagonists (trifluoperazine, W-7, promethazine) inhibited, in a dose-dependent manner, spontaneous repeated hyperpolarizations. Trifluoperazine completely inhibited the hyperpolarization induced by the Ca^{2+} or Sr^{2+} injection, but not the calmodulin-induced hyperpolarization. These observations are consistent with the hypothesis that calmodulin is involved in the opening of the Ca^{2+}-activated K^+ channels in L cells.

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IMMOBILIZATION OF GATING CHARGES IN THE NA CHANNEL OF CRAYFISH GIANT AXONS.
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To clarify the molecular mechanisms underlying the relation between the activation and inactivation processes in Na channels, effects of synthesized pyrethroids, p-SO_2Me ($1x10^{-4}M$) and o-SO_2Me ($1x10^{-3}M$) were studied under voltage clamped conditions on Na conductance changes obtained from intact axons and also on asymmetrical capacitance currents (I_s) obtained from internally perfused axons. Both compounds reduced the peak amplitude of Na currents (I_{Na}) without affecting the rate of rise of I_{Na}. o-SO_2Me slowed the time course of Na inactivation process with no tail currents after repolarization to E_K. In the axons treated with p-SO_2Me, a fraction of the affected channels was never inactivated leaving a residual I_{Na} (suggesting that $\alpha_h \neq 0$) accompanied with a marked inward tail current after repolarization. Experiments with I_s showed that both compounds greatly decreased the amount of gating charges displaced on depolarization (Q-on). o-SO_2Me partially restored the decrease in amplitude of I_s-off that was normally brought about with prolonged depolarizing pulses, while p-SO_2Me diminished I_s-off to almost unmeasurable size. The results suggest that the effect of stabilizing the gating charges of activation to a channel-open position is closely linked to a mechanism that inhibits Na inactivation to proceed further.

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THE RANGE OF SODIUM CHANNEL-ACTIVATION BY N-ACETYLIMIDAZOLE AND ITS PERSISTENCE IN GLUTAMATE INDUCED RESPONSES OF THE GLUTAMATE-HYPERPOLARIZATION TYPE SNAIL NEURONES. MARUHASHI, J. and KUSANO, K. Dept. of Biology, Daiichi College of Pharmaceutical Sciences, Minami-ku, Fukuoka 815 and Dept. of Biology, Illinois Institute of Technology, Chicago 60616, USA.

ACh or Glu induced depolarizing responses due to Na^+ permeability increase are depressed with application of N-acetylimidazole (NAI) in snail D-type neurones, irreversibly. In Glu-H type neurones (Oomura et al., J. Physiol.,243:321-341, 1974; Kerkut et al., Comp. Biochem. Physiol., 50A:1-25, 1975), Glu induced responses had no Na^+ ΔG increase responses. During perfusion of NAI, Glu induced responses in the Glu-H type neurones had Na^+ channel activated depolarization with increase of ΔG in the range from 0.1 to over 0.8 µS. NAI-activated depolarization had the duration over one hour with two min application of Glu. The effect of NAI on Na^+channel activation persisted over two hours. These NAI-activation of Na^+channel in the Glu-H type neurones were reversible. But, in the "low NAI-activation" neurones, NAI activated responses were depressed with every NAI-applied time, irreversibly.

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PHARMACOLOGICAL PROPERTIES OF K-CHANNELS ACTIVATED BY AN INCREASE IN EXTERNAL K^+-CONCENTRATION IN MOUSE NEUROBLASTOMA CELLS MIYAKE, M., NAKATA, H. AND KURIHARA, K. Fac. of Pharmaceu. Sci., Hokkado Univ., Sapporo

We have previously reported that mouse neuroblastoma cells (clone N-18) carry the K-channels activated at high $[K^+]_o$ (>10 mM). To explore the properties of the K-channels, the effects of various agents on the ^{86}Rb efflux induced by an increase in $[K^+]_o$ were examined. 1) An increase in ^{86}Rb efflux by elevating $[K^+]_o$ was unaffected by 5 mM tetraethylammonium or 0.1 mM 4-aminopiridine which suppressed appreciably the voltage-dependent K-channels of N-18 cells. 2) Apamin (up to 1 µm), a blocker of the Ca-dependent K-channels, had no effect on the increase in ^{86}Rb efflux, while quinidine (0.1 mM), another blocker, suppressed it. This blocking effect, however, may be stemming from its non-specific effect as well as that of local anethetics such as procaine. 3) The increase in ^{86}Rb efflux was observed even in the absence of external Ca^{2+}. The elevation of $[Ca^{2+}]_o$ inhibited the efflux increase in a seemingly competitive mammer to $[K^+]_o$. The other polyvalent cations exhibited a similar blocking effect. The inhibitions can be explained in terms of their shielding effect on K^+ getting close to the membrane surface. The above results strongly suggest that the increase in ^{86}Rb efflux by elevation of $[K^+]_o$ was mediated by novel K-channels activated by the binding of K^+ to it.

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EFFECTS OF INTRACELLULAR DIVALENT CATIONS ON Na CHANNEL AND K CHANNEL ACTIVATIONS OF SQUID GIANT AXONS. YAMAGISHI, S. and FURUYA, K. Dept. of Cell Physiology, National Institute for Physiological Sciences, Myodaiji, OKAZAKI 444

The membrane current of squid giant axons immersed in artificial sea water was measured under intracellular perfusion of a K-salt solution at the concentration ranging 25 to 250 mM as a control. Then, the effects of various divalent cations, such as Mg, Sr, Co, Mn, Ni and Zn ions, on the inward current (I_{Na}) and the outward current (I_K) were examined compared with that of Ca ion immediately after the onset of internal pefusion. By addition of 1mM or 4mM divalent cation, the initiation of I_{Na} shifted to lower voltage by 5∿15mV within 60sec after the onset of internal perfusion in all species. At the same time, I_{Na} was suppressed by 5 to 40% of the control. I_K was also suppressed by the addition of divalent cations more than by 10%. According to the degree of I_K suppression, divalent cations could be classified into three groups, namely, (1) Ca ion; the degree of I_K suppression was fewer than that of I_{Na}, (2) Mg, Sr, Co, Mn and Ni ions; the degree of I_K suppression overcame that of I_{Na} and (3) Ba and Zn ions; I_K was suppressed nearly to zero.

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DEVELOPMENT OF TETRODOTOXIN SENSITIVE SODIUM ACTION POTENTIAL IN CULTURED L6 MYOTUBES. YAMAZAKI, S., SATOH, T., KANO, M. AND YAMAMOTO, M. Department of Physiology, School of Medicine, Kitasato University, Sagamihara 228

Tetrodotoxin(TTX) usually blocks the action potential (AP) in adult vertebrate innervated skeletal muscle, i.e. the AP is operated by a TTX-sensitive Na channel. The L6 cultured myotube, however, has TTX-resistant AP (Kidokoro, 1973) that is usually found in skeletal muscle following denervation or immediately after birth in rat. The TTX-resistivity of the L6 myotubes might be due to a lack of a neurotrophic substance. The present study revealed that brain extract had a trophic substance and could induce the TTX-sensitive Na channels in uninnervated or never-innervated cultured L6 myotubes. 18-19 days old cultures were used and the maximum rate of rise of AP was measured as an index of the Na channel density. In the result, the values of maximum rate of rise of AP in control cultures were about 120 V/sec and did not change by TTX application, indicating that the Na channel in the control culture is TTX-resistant. However, in cultures with the brain extract the values were 142 V/sec and reduced to 86 V/sec in the presence of TTX. The reduced maximum rate of rise of AP by TTX application in the latter cultures means that the brain extract produced TTX-sensitive Na channels in L6 myotubes. Regarding to the development of the sodium channels, the L6 cells could well differentiate in culture without innervation, but in the presence of brain extract.

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PARTIAL PURIFICATION OF THE PHOTOLABELED PROTEIN WITH AN AZIDE DERIVATIVE OF TETRODOTOXIN IN *ELECTROPHORUS electricus* ELECTROPLAX MEMBRANES. UEHARA, S. and UYEMURA, K. Dept. of Physiol., Saitama Med. Sch., Moroyamacho, Iruma-gun, Saitama 350-04.

A tritiated azide derivative of tetrodotoxin (TTX), 2-nitro-4-azidephenyl-(^{3}H)-β alanine-TTX (890 mCi/mmol), was synthesized and identified with reverse phase high performance liquid chromatography. TTX binding sites of *Electrophorus electricus* electroplax membranes were photolabeled with the ligand at the concentration of 20 - 50 nM in the presence or absence of TTX. The photolabeled protein was solubilized with 1% Lubrol PX and applied on Sephadex G 25 column (15 x 400 mm) and eluted with 0.1% Lubrol-50 mM ammonium acetate buffer. The labeled protein was eluted with an elution volume greater than the total bed volume of the column (Kav=2.1), and purified further on Sepharose 6 B column (15 x 900 mm, 1% SDS-50 mM ammonium acetate buffer). The purified protein showed the molecular weight of around 10,000 and higher specific activity of up to 500 times (350 pmoles of the ligand/mg protein) than that of the membranes. Further purification and characterization of the protein are in progress.

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SYNTHESIS OF TETRODOTOXIN DERIVATIVE AND PURIFICATION OF TETRODOTOXIN BINDING PROTEIN FROM E. ELECTRICUS ELECTROPLAX. NAKAYAMA TAKASHI and SUSUMU TERAKAWA Dept. of Cell Physiology, National Institute for Physiological Sciences Myodaiji, Okasaki 444

Tetrodotoxin (TTX, citrate-free) was coupled with biotin (vitamin H), and the coupled product was purified by high performance liquid chromatography on a reverse-phase column essensially same as described previously (Nakayama and Terakawa, Anal. Biochem. 126, 157 1982). Purified biotinyl-TTX retained its toxic activity. Na-channel enriched membrane was prepared from electroplax. The membrane was pre-incubated with biotinyl-TTX, and then solubilized with Lubrol-PX in the presence of phosphatidylcholine. After centrifugation at 100,000 x g for 1 h, the supernatant containg TTX binding protein was applied on an avidin conjugated agarose column. After the column was washed, TTX binding protein was eluted with excess TTX. The recovered eluent was analyzed by SDS-polyacrylamide gel electrophoresis. The gel pattern indicated that about 250,000 dalton polypeptides were major components of TTX binding protein.

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INFLUENCES OF CONDUCTING STATE OF Na CHANNELS ON ACTIONS OF ACONITINE AND SEA ANEMONE TOXIN. WARASHINA, A Dept. of Physiol., Niigata Univ. Sch. of Med., Asahi-machi, Niigata 951

Aconitine was applied to crayfish giant axons under voltage-clamped conditions. Unlike its action on myelinated nerve fibers, aconitine did not enhance the Na conductance in the crayfish axon at membrane voltages near the resting potential but simply reduced the Na current in the entire range of I-V relation. The aconitine block took place in a frequency-dependent manner, i.e., the blocking developed only under repetitive activations of Na channels by depolarizing pulses. The duration of pulses, either 2 ms or 10 ms,did not affect the blocking rate. The final extent of block increased at higher frequency and more positive voltage of pulses. The Na current was suppressed by about 70 % with 50 μM aconitine under repetitive pulses to -20 mV at 0.5 Hz from holding potential (-100 mV). After inhibition of the normal inactivation by treating axons with sea anemone toxin from Parasicynois actinostoloides, aconitine blocked the Na current in both frequency- and duration-dependent manners. These results suggest that aconitine binds to Na channels from inside the axon to interfere with the permeability of Na ions, the affinity of the reaction presumably depending on the electrochemical potential of aconitine molecules.

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RAPID CHANGES IN INTRACELLULAR PRESSURE OF SQUID GIANT AXONS.
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Rapid changes in intracellular pressure of the squid giant axon were measured by an optical fiber device. When the action potential was produced upon electrical stimulation, the intracellular pressure rose and then fell in a few milliseconds. The amplitude of the pressure response was about 10 mPa in intracellularly perfused axons and about 1 mPa in unperfused axons. The falling phase was enhanced greatly when the axon was immersed in a medium containing high concentration of Ca-ion (100 mM). The time course of the falling phase became very slow at a low temperature (7°C). Pressure responses with a long rising phase were found to be associated with plateau-type action potentials produced under the effect of tetraethylammonium, low ionic strength, or proteolytic enzyme. Depolarization of the membrane by a rectangular pulse of current resulted in a rise in intracellular pressure, and hyperpolarization in a fall in intracellular pressure. Lidocaine applied externally suppressed these pressure responses completely. The pressure response observed with the voltage clamp pulse depended parabolically on the membrane potential. The diameter of the axon increased when the intracellular pressure rose. From these findings, it is tentatively concluded that the mechanical responses in nerve fibers are due to potential dependent variations in thickness of the phospholipid membrane.

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FACILITATION OF GABA- AND PENTOBARBITAL (PB)-INDUCED Cl CURRENTS BY PB AND GABA.
HATTORI, K., KURAOKA, S., AKAIKE, N. AND OOMURA, Y. Dept. of Physiol., Kyushu Univ., Faculty of Med., Fukuoka 812

Cl currents in GABA-sensitive neurons of the bullfrog dorsal root ganglion were separated from other Na, K and Ca currents using a suction pipette technique which allows internal perfusion and voltage clamp. Both GABA and PB evoked Cl currents dose-dependently. PB-induced maximum Cl current was about half of GABA-induced one. The number of drug molecules required to activate a receptor (n) was 1 for GABA responses and 2 for PB responses. In the presence of 10^{-6}M GABA, PB-induced currents were maximally enhanced by about 10 times, the maximum current almost became equal to the GABA-induced maximum current, and the dose-response curve gave $n = 2$. The enhancement was also voltage-dependent, though the greater enhancement was obtained in less positive membrane potentials than Cl equilibrium potential regardless of different Cl equilibrium potentials at various external and internal Cl concentrations.

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γ_{Cl} IN GABA-SENSITIVE FROG DORSAL ROOT GANGLION CELLS.
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The experiments were performed on isolated neurons in the lumber dorsal root ganglion of the bullfrog. The Cl currents in the nerve cell body were separated from other Na, K and Ca currents by suction pipette technique which allows voltage clamp and intracellular perfusion. GABA- and pentobarbital (PB)-induced Cl current fluctuations were analyzed by power-spectrum. Each drug gave at least two corner frequencies. The lower corner frequency was clearer than the higher one at low drug concentration while the higher corner frequency became more clear at the high concentration. 10^{-6}M GABA and 10^{-4}M PB, at which concentrations there were no desensitizations, gave 10 to 20 pS. The values were almost equal to the γ_{Cl} calculated by amplitude histogram analysis. The γ_{Cl} values in the presence of GABA or PB were also quite close to those calculated from the slope of the current-voltage relationships obtained in the inside-out patch clamp recordings. In addition, the histogram of open time of GABA- and PB-induced single channel currents did not show simple exponential form. It may confirm the results of power-spectrum analysis.

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EXTERNAL AND INTERNAL PERFUSION OF GABA-ANTAGONISTS.
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The Cl currents in the frog dorsal root ganglion cells were separated from Na, K and Ca currents by using internal perfusion technique. The dose-response curves to GABA were investigated in the presence of external and internal application of GABA antagonists. With external application of antagonists, the inhibition of the GABA-induced Cl currents by bicuculline, strychinine, curare, atropine, D-600 and SHIT was competitive, that by picrotoxin was noncompetitive, while that by penicilline was uncompetitive. Only picrotoxin blocked GABA and pentobarbital (PB) responses from either side of the cell membrane. Picrotoxin changed the slope of a ramp current-voltage relationship induced by GABA and PB, and also blocked the single Cl channel currents in the inside-out patch clamp preparations. Unexpectedly, PB-induced responses were blocked by picrotoxin with the reduction of mean channel conductance and shortening of lifetime of single Cl channel currents.

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KINETIC ANALYSIS OF THE ACTION OF CATECHOL ON THE NEUROMUSCULAR JUNCTION.

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A tremendous enhancement with 4-aminopyridine (4AP) of the amount of acetylcholine (ACh) released by a single nerve impulse leads to a depletion of the readily releasable ACh. Consequently, unless readily releasable ACh quanta are recruited from depot, subsequent stimulations of the motor nerve fiber give rise to much diminished ACh releases compared with the first one. Such a diminution in the ACh output, known as the early tetanic rundown of endplate potential or the depletion of transmitter, has been adopted in analyzing the kinetics of actions of various release-modifying agents. During these studies, we found that several aromatic compounds such as catechol (pyrocatechol, PC) and phenylenediamines facilitate the transmitter release. The presynaptic action of PC was especially prominent. The dose-release study revealed that PC was only four times less effective than well-known 4AP but was much stronger than noradrenaline, guanidine and tetraethylammonium. In the present experiments, the effect of PC was found to be identical in many respects to the case of 'chemical potentiation or accelerated evoked release' of 4AP. A synergistical action of Ca ions and an antagonistic interaction of streptomycin were the same as those observed with 4AP. As seen with 4AP, moreover, PC at the concentration as low as 50 μM prolonged the nerve terminal action potential.

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EFFECTS OF 5-HYDROXYTRYPTAMINE ON ACCUMULATION OF CYCLIC AMP IN THE VENTRICLE AND ANTERIOR AORTA OF APLYSIA.

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The effects of 5-hydroxytryptamine (5-HT) on the accumulation of cyclic AMP (cAMP), on tension and on $^{45}Ca^{++}$ efflux in the ventricle and anterior aorta of Aplysia kurodai were studied. 5-HT produced tension increases and phasic contractions in the anterior aorta and has positive inotropic and chronotropic effects on Aplysia heart. An analogue of cAMP, 8-butyryl cAMP (8-BT cAMP) mimicked the effect of 5-HT on the ventricle. 5-HT application caused an increase in cAMP levels in the ventricle and anterior aorta with a threshold of between 10^{-8} and 10^{-7}M and 60-100 fold increases at 10^{-4}M 5-HT. The phosphodiesterase inhibitors, theophylline and 3-isobutyl-1-methylxanthine (IBMX) mimicked the effect of 5-HT on the heart muscle, causing dose-dependent increases in heart rate and tension. Incubation of the ventricle and anterior aorta in 10^{-7}M to 10^{-4}M 5-HT elecited a dose-dependent, transient increase in the efflux of preloaded $^{45}Ca^{++}$. Efflux of $^{45}Ca^{++}$ was also induced by 8-BT cAMP, caffeine and glycine. These results suggest that 5-HT stimulates production of cAMP which mediates the effects of this amine on the ventricle and anterior aorta of Aplysia by influencing intracellular Ca^{++} concentration.

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BLOCKING ACTION OF SEROTONIN ON THE INHIBITORY DOPAMINE RECEPTOR OF THE APLYSIA GANGLION CELL.

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The application of dopamine(DA) on a certain ganglion cells of Aplysia elicits a hyperpolarizing response with a marked increase in permeability of the receptor membrane toward K^+-ion. This inhibitory response was evaluated by the change in membrane conductance (ΔG) using modulated resting clamp method. A 2 minute exposure to 1 μM 5-HT significantly depressed the inhibitory responses to 10 μM DA. The depressing effect of 1 μM 5-HT was completely reversible after 15 minute washing. A mode of blockade was studied by plotting dose-inhibition curve and Lineweaber-Burk type for receptor activity. The systematic shift of these curves indicated the blockade was "competitive". It was concluded that 5-HT competes with DA for a common binding site at the DA receptor of this type.

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THE NATURE OF MONOAMINE-INDUCED RESPONSES IN THE LATERAL HORN CELL OF CAT SPINAL CORD IN VITRO. INOKUCHI, H., HIGASHI, H., YOSHIMURA, M. and NISHI, S. Dept. Physiol., Kurume Univ. Sch. Med., Kurume.

Intracellular recordings were made from neurons in the lateral horn of the thoracic cord transversely sectioned at about 400 μm intervals. Noradrenaline (NA) and 5-hydroxytryptamine (5-HT) at concentrations of 10 to 100 μM caused in the majority of neurons a slow depolarization associated with an increased membrane resistance. The depolarizing response was unaffected by reduction of external Na or Cl, while it was enhanced in a low K medium. The response was augmented by depolarization whereas it was eliminated by hyperpolarization exceeding about -70 mV. These characteristics suggest that the depolarizing response is caused by closing of the K channels which resemble the 'M' channels of ganglion cells. NA induced in the minority of neurons a slow hyperpolarization accompanied by a reduced membrane resistance. The hyperpolarizing response had its equilibrium potential at about -90 mV which was independent of external Na or Cl concentration. It should be added that NA abolished the slow afterhyperpolarization which is due to a Ca-activated G_K. The NA-induced hyperpolarization is therefore seemed to be generated by an increased resting G_K. These results suggest that NA and 5-HT are not the transmitter mediating the fast EPSP but they may mediate the slow EPSP, and that NA may produce a slow IPSP in some neurons.

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AN ANALYSIS OF THE TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION TREATED WITH XANTHINE DERIVATIVES. TAKEUCHI, N., TAKIKAWA, Y. and YANA, K. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo, Tokyo 113

Spontaneous miniature end-plate potentials (m.e.p.p.) were recorded at the frog neuromuscular junction. Theophylline (0.5-2mM), one of the xanthine derivatives, induced an increase in frequency of the m.e.p.p. and spontaneous giant potentials (s.g.p.) with amplitudes of more than three times the modal value of m.e.p.p. These effects of theophylline were reversible and were not remarkably affected by changing the external ion composition within the physiological range. Theophylline merely shifted the log $[Ca]_o$ vs. log m.e.p.p. frequency curve upward. The results indicate that the effects of theophylline are caused mainly by a change at an intracellular site that controls the release mechanism.

The s.g.p. showed a positive correlation between the rise time and the peak amplitude, and notches were frequently observed in the rising phase. Statistical tests for Poisson process were applied to fairly long m.e.p.p. occurrence time sequences. Sherman's statistics showed significantly large values under the treatment of theophylline, indicating that the time sequences had a tendency to cluster. This statistical analysis together with electrophysiological findings implies that the s.g.p. might be due to the clustering of the m.e.p.p.

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THE K CHANNEL COUPLED WITH THE MUSCARINIC ACh RECEPTOR IN THE HEART MUSCLE. NOMA, A and SOEJIMA, M* Natl. Inst. Physiol. Sci., Okazaki, 444 Dept. Int. Med, Jikei Univ. Sch. Med., Tokyo, 105

In order to examine whether intracellular mechanisms are involved in opening the K channel by the muscarinic receptor, the patch clamp technique was applied to single cells enzymatically isolated from the rabbit atrium. Control (150 mM KCl) and test solutions were perfused through a thin tubing within the electrode pipette with a pressure of -40 cm H2O whilst recording. In the absence of ACh, the K channel opened at a low frequency (1-50/sec). The channel showed a mean life time of 1-2 msec and a conductance of 50 pS for the inward flow, less conductance for the outward flow. When ACh was applied in the bath, the channel activity was not affected. After washing out ACh, perfusion of the pipette with 0.01 to 1 μM ACh largely increased the opening probability within 1-2 min. When the pipette solution was switched from the control to 1 μM atropine solution, the activity was depressed. These results indicate direct coupling of the K channel with the muscarinic reptor.

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MODULATORY ACTION OF BIOGENIC SUBSTANCES ON THE DESENSITIZATION OF NICOTINIC ACH RECEPTOR. YHUKOU OHTA, TAKASHI AKASU and KYOZO KOKETSU. Dept. of Physiology, Kurume University School of Medicine, Asahi-machi, Kurume 830.

Desensitization is known as a diminution of the receptor sensitivity during a continuous or repetitive application of an agonist. When repetitive iontophoretic applications of ACh were applied to the frog end-plate, ACh potential or current diminished its amplitude and then reached a certain steady state level within a few minutes. 5-HT (0.1 mM), isoproterenol (0.1 mM), LHRH (4.6 μM) and substance-P (4.2 μM) increased the rate of desensitization and increased desensitized receptors. A biphasic time course of desensitization onset was observed in the presence of LHRH and substance-P; it composes fast and slow exponential phases. The recovery of desensitization was not significantly altered by 5-HT, isoproterenol or LHRH. On the other hand, biphasic recovery from desensitization was observed in the presence of substance P. These results suggest that some neurotransmitters and hormone may be playing a significant role for the modulation of synaptic transmission by modulating the desensitization of postsynaptic receptors.

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MODULATORY EFFECT OF LUTEINIZING HORMONE-RELEASING HORMONE AND SUBSTANCE P ON THE NICOTINIC TRANSMISSION IN BULLFROG SYMPATHETIC GANGLIA. TAKASHI AKASU, MIKIE KOJIMA† and KYOZO KOKETSU. Dept. of Physiol., Kurume Univ. Sch. Med., Kurume 830 & Dept. of Chem. Fac. Sci., Kyushu Univ., Fukuoka 812†.

Luteinizing hormone-releasing hormone (LHRH) and substance P are endogenous polypeptides which are considered to be neurotransmitters of non-cholinergic postsynaptic potentials in various autonomic ganglia. ACh current produced by iontophoretic application of ACh to voltage-clamped sympathetic ganglion cells and skeletal muscle end-plates were markedly depressed by LHRH and substance P in low concentrations (0.1 - 5 μM). According to the analysis with Michaelis-Menten type kinetics, LHRH and substance P depressed the maximum response (Vmax) of the dose-response curve of ACh current without changing the affinity (Km) of ACh to the receptor. These peptides altered neither the reversal potential nor time course of the end-plate current. LHRH and substance P (100 pM - 100 nM) markedly increased the amplitude and quantal content of fast e.p.s.p., while those in concentration more than 0.5 μM depressed the amplitude and quantal content of fast e.p.s.p. From these experimental results, it is suggested that LHRH and substance P modulate the cholinergic transmission by controlling not only the sensitivity of nicotinic ACh-receptor at the subsynaptic membrane but also the evoked release of ACh from presynaptic nerve terminals.

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EFFECTS OF 5HT AND GABA ON SPONTANEOUS ELECTRICAL ACTIVITY OF A PEPTIDE NEUROSECRETORY SYSTEM. NAGANO, M. Medical Research Institute, Tokyo Medical and Dental University, Tokyo, 101

Electrical activity of the crab (Cardisoma carnifex, Podophthalmus vigil) peptidergic neurosecretory system, the X-organ-sinus gland, was monitored extracellularly from the axon tract simultaneously with an intracellular recording from a soma or from a terminal. The isolated system was mounted in a double chamber permitting continuous, separate perfusion of the X-organ region (XO, somata and axon collaterals) and sinus gland (SG, terminals). 5HT, added to the saline perfusing the XO, resulted in a dose-dependent (10^{-6} - 10^{-4} M) inhibition of intracellularly recorded spontaneous activity of terminals of Podophthalmus. In responsive somata, hyperpolarization was observed, as well as decreased firing rate. 5HT had no consistent effects when perfused on SG. GABA, examined in Cardisoma, inhibited or silenced spontaneous activity; concentrations of >0.5 X 10^{-3} M were required. Some recordings from somata showed a transient, small hyperpolarization. In terminals it resulted in depolarization (to about -50 mV). The observations are consistent with a role of 5HT and GABA in synaptic activation of the neurosecretory cells; the post-synaptic sites would occur on collaterals. The experiments were done at the Békésy Lab., U. of Hawaii, supported by NIH Grant NS 15453 to I. Cooke.

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EFFECTS OF BATH-APPLIED EXCITATORY AMINO ACIDS AND THEIR ANALOGUES ON SPINAL GIANT INTERNEURONS OF THE LAMPREY. HOMMA,S. and SEKIYA,S.* Dept.Physiol., Fac.Med., Toyama Med.Pharmaceut. Univ. Toyama 930-01 and Saigata National Sanatorium.

Conductance increases(ΔG) produced by glutamate(glu), aspartate(asp), DL-homocysteate(Hom) were reduced to 10-30 % after blockade of synaptic transmission in TTX(0.5 μg/ml), 4-aminopyridine(1 mM) and Ca^{++}-free perfusate(TTX-sol.). In contrast, depolarizations(ΔV) by these agonists were increased by 30-50 % after blockade of transmission. These results were also seen in low-Cl^- perfusate(10 %). These observations suggest the large contribution of inhibitory transmitter to the responses by these agonists in normal perfusate(2.6 mM Ca). Usual ΔG increases by N-methyl-DL-aspartate(NMDLA) in normal sol. were converted into depolarizations associated with apparent ΔG decreases in a dose-dependent fashion in TTX-sol.. Reversal potentials(Vrev.) estimated from V-I curves obtained in the presence and absence of glu or asp were around -20 mV, suggesting the increases of G_{Na} and G_K simultaneously by glu and asp. In contrast, Vrev. for NMDLA was around -90 mV, suggesting the decrease in G_K. Dose-response curves of glu and asp in TTX-sol. gave Hill coeffcient around 2 both for ΔG and ΔV as in the case of iontophoresis. Time courses of ΔV of 1 mM glu and asp(20 ml)(flow rate=10 ml per min) were similar and 1.4 min in time to peak and 2.5 min for recovery. Those by Hom, NMDLA, quisqualate and kainate were about 2 times longer than those by glu and asp.

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PHARMACOLOGICAL SENSITIVITIES OF AMINO ACID RESPONSES IN A PREPYRIFORM BRAIN SLICE. HORI, N., NAKANISHI, H., FFRENCH-MULLEN, J. and CARPENTER, D. O. Dept. of Pharmacology, Faculty of Dentistry, Kyushu University, Fukuoka 812 and New York State Dept. of Health, Albany, New York

The lateral olfactory tract (LOT) forms excitatory synapses on pyramidal neurons of the prepyriform cortex, and is thought to utilize aspartate as a transmitter. In a rat brain slice preparation we have compared the pharmacologic sensitivity of the LOT potentials with those to ionophoretic application of excitatory amino acids. Glutamate diethylester and aminoadipate, presumed specific antagonists of glutamate and aspartate receptors, respectively, had essentially no effect on responses to LOT stimulation or application of aspartate, glutamate, quisqualate or N-methyl-DL-aspartate (NMDA). Amino-phosphonobutyric acid (10^{-4} M) blocked the effects of LOT stimulation but not those of aspartate, glutamate or NMDA. Responses to aspartate, glutamate and quisqualate were associated with an increase in membrane conductance while that to NMDA was associated with a conductance decrease. These results indicate that none of these amino acids is the LOT transmitter or activates the synaptic receptor of the LOT.

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GABA-ACTIVATED Cl CHANNELS IN RAT HIPPOCAMPAL NEURONS IN DISPERSED CELL CULTURE.
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Hippocampal neurons derived from 17- to 19-day-old fetal rats have been grown in a monolayer culture. Some of the neurons developed a characteristic branching pattern resembling that of hippocampal pyramidal cells in vitro. These neurons were sensitive to GABA and glutamate before acquisition of synaptic input. The properties of the GABA-activated channels in the neurons were studied with the patch electrode voltage clamp technique (Hamill et al., Pflügers Arch. 391 : 85, 1981). Application of GABA ($> 2\,\mu$M) caused an increase in the membrane permeability exclusively to Cl ions. The power density spectrum during the steady state GABA current could be fitted with two Lorentzians of which corner frequencies were 3.8 ± 0.3 and 24.8 ± 4.0 Hz (mean ± S.E., n=5) at 30°C. The effective single channel conductance estimated from the mean current and the variance of current fluctuations was 18.9 pS, when the intra- and extracellular Cl^- concentrations were 150 and 120 mM, respectively.

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Effects of 2-amino-4-phosphonobutyric acid on synaptic transmission between mossy fibers and CA3 neurons in the guinea pig hippocampus. Sawada, S. and Yamamoto, C. Dept. of Physiology, Faculty of Medicine, Kanazawa University, Kanazawa 920

The purpose of this study was to examine whether 2-amino-4-phosphonobutyric acid (APB) blocked mossy fiber-induced excitation in CA3 neurons in thin sections of the guinea pig hippocampus. D- and DL-APB suppressed field potentials induced in regio CA3 by granular layer stimulation. Threshold concentration of DL-APB to induce the suppression was 2-5 u̲M. D-APB was about 10-fold less potent than DL-APB. Field potentials induced by fimbrial stimulation were much less affected. DL-APB suppressed excitatory postsynaptic potentials recorded intracellularly from CA3 neurons in response to granular layer stimulation but caused no marked changes in resting potentials, action potentials and membrane conductance. Single cell discharges induced by iontophoretic administration of glutamate (Glu) or aspartate (Asp) were unaffected when mossy fiber-induced excitation was suppressed by D- or DL-APB. DL-APB had no suppressing action on Glu- or Asp-induced depolarizing potentials. It was concluded that APB was a relatively specific blocker of synaptic transmission between mossy fibers and CA3 neurons, and that this action did not result from blockade of receptors for Glu or Asp.

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EFFECTS OF OXYGEN AND GLUCOSE DEPRIVATION ON THE NEURONAL ACTIVITY AND THE LEVEL OF HIGH ENERGY PHOSPHATES IN THIN HIPPOCAMPAL SLICES. OKADA, Y. and YONEDA, K. Dept. Physiology, Kobe Uinversity, School of Medicine, Chuo-ku, Kobe,650.

Effects of anoxia were studied using thin hippocampal slices from the guinea pig. Electrical stimulation to the granular cell layer of the dentate gyrus elicited the postsynaptic field potentials(PSP) in the pyramidal cell layer(CA_3 and CA_4 area) of the hippocampus. Concentrations of ATP and P-creatine in the slices not exposed to anoxia were 13.2 and 28.3 mmol/kg protein respectively. Deprivation of both oxygen and glucose in the perfusion medium reduced the amplitude of PSP and blocked it completely within 7 min(half time; 4 min). The level of tissue ATP and P-creatine decreased rapidly(half time; 3 and 5 min). During re-oxygenation(oxygen and glucose), electrical activity and the high energy phosphates recovered after 10 min anoxia but no complete recovery was found thereafter. Deprivation of only oxygen reduced the PSP slowly with an initial slight increase in the amplitude and blocked it in 45-60 min. Tissue ATP and P-creatine were 70% and 40% of the original level even at 60 min after the onset of anoxia. During re-oxygenation the PSP recovered even after 60 min anoxia with good recovery in the level of high energy phosphates. Thus the presence of glucose during anoxic episode is a critical factor for the neuronal activity and the preservation of high energy phosphates during anoxia and during recovery from anoxia.

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EFFECT OF POLYVALENT CATIONS ON THE GLUTAMATERGIC SYNAPSE AT INSECT NEUROMUSCULAR JUNCTIONS. MIYAMOTO, T* and WASHIO, H. Lab. Neurophysiol., Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194.

The effect of extracellular polyvalent cations on the glutamatergic neuromuscular transmission was studied in the skeletal muscle fibers of the mealworm, Tenebrio molitor. It was found that some divalent cations such as Ni^{2+}, Co^{2+} and Mn^{2+}, which are known as Ca^{2+}-antagonist, decreased the neurally evoked excitatory postsynaptic potential (EPSP) but increased the response to L-glutamate (L-GA) applied ionophoretically (GP). The external concentration of Ca^{2+} and Mg^{2+} did not have any effect on the GP. Since the apparent maximum of the dose-response curve for GP changed in the presence of these divalent cations, it seemed unlikely that the cations competed with L-glutamate for a common binding site on the postsynaptic membrane. Under a two electrode voltage-clamped condition, these divalent cations did not change the reversal potentials of the excitatory postsynaptic current (EPSC) and the current recorded during L-GA iontophoresis (GC). Furthermore, these ions increased the time constant of decay of EPSC and GC. These results suggest that these transition metal ions affect the sensitivity of the postsynaptic membrane to L-GA by modulating the kinetics of the less ion-specific channels coupled with the GA receptor, in addition to prejunctional action of these ions mediating with antagonism to Ca^{2+}.

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ELECTRICAL PROPERTIES AND CHEMOSENSITIVITIES OF THE DORSAL RAPHE NEURONES OF THE RAT.
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Intracellular recordings were made from neurones in the dorsal raphe nucleus (DRN) in tissue slices transected from rat's brain. DRN neurones were characterised by high input resistances (50-400 MΩ), long membrane time constant (4.0-32.2 ms) and short electrotonic length of their dendrites (length/space constant, 0.4-0.9). Fast EPSPs which are most likely mediated by l-glutamate were dose-dependently depressed by noradrenaline (NA, 1-100 μM) or enkephalin (ENK, 0.1-1 μM). Since NA and ENK did not appreciably alter the l-glutamate-induced depolarization of DRN neurones, their depressant action on fast EPSPs is thought to be presynaptic. In addition to such a presynaptic action, NA or dopamine (DA, 10-100 μM) caused in many neurones a slow depolarization associated with an increased membrane resistance. The NA- or DA-produced depolarization was depressed by phentolamine (1 μM) but unaffected by propranolol (1 μM), suggesting that the NA and DA actions are mediated by an alpha receptor. ENK, on the other hand, often generated a hyperpolarization associated with a decreased membrane resistance. Thus the pre- and postsynaptic ENK action appeared to be solely inhibitory. The substances so far confirmed to be postsynaptic excitants were homocysteic acid, l-glutamate, aspartate, NA, DA and acetylcholine in the order of potency.

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DIFFERENT MODE OF ACTION OF EXCITATORY AMINO ACIDS IN PLOTOSUS ELECTRORECEPTORS.
NAGAI, T. AND OBARA, S. Dept. of Physiol., Sch. Med., Teikyo Univ., Tokyo 173

In ampullary electroreceptors of Plotosus, the spontaneous afferent discharges are due to continual release of the natural transmitter, and can be completely suppressed simply by hyperpolarizing the ampulla. L-glutamate (Glu) iontophoretically applied to thus suppressed ampulla proved to excite a single afferent postsynaptically in a dose-dependent manner. L-aspartate (L-Asp), in addition to weak excitatory action, markedly potentiate the Glu responses, when applied through multi-barrelled electrodes. In the present experiments several amino acids were further examined. Cysteate (CA) and also cysteine sulfinate (CSA) induced single unit discharges. The response pattern of these L-Asp analogues was similar to that of L-Asp both in their latency and time course, and clearly different from that of Glu. Furthermore, when applied as conditioning, CA, CSA and D-Asp even with their subthreshold doses could potentiate the Glu responses. As was observed with L-Asp, such potentiation could last long after a relatively short conditioning pulse of these analogues. Homocysteate was only weakly excitatory, and kainate was stronger and more persistent compared with Glu. No potentiation, however, was observed with either of them.

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CONTRIBUTION OF RECEPTOR CELL ACTIVITY TO A STEADY EPITHELIAL CURRENT IN PLOTOSUS ELECTRORECEPTORS.
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The regular "resting" afferent discharges of Plotosus electroreceptors in situ have been assumed to result from an epithelial current flow in the sensory epithelium (ampulla) which is electrically shunted through a low-impedance path (its own duct). In order to simulate such in situ conditions and to measure the epithelial current, a variable electronic shunt circuit was applied to isolated ampullae. The in situ response pattern was reversibly reproduced by a shunt resistance of c. 50 KΩ at a DC level near 0 mV. The epithelial current showed a negative slope property, consisting of a passive bias current and active components (I_{Ca} & I_K) in the basal membrane of receptor cells. At the in situ DC level the net epithelial current was close to zero with a small continual I_{Ca}. The peak I_{Ca} on perturbation was alswys smaller than that of V-clamped ampullae. The maximum active slope (g_{Ca}), however, remained unchanged, and the apparent E_{Ca} shifted to lower levels. Simultaneous PSP recording indicated a steady afferent output at rest, which steeply increased on small increments in I_{Ca}. Thus, the shunted and biased sensory epithelium apparently provides the basis for the high-gain synaptic transfer in the present sensory receptor.

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EFFECTS OF ELEVATED TONICITY ON TETANIC POTENTIATION OF MINIATURE END-PLATE POTENTIAL FREQUENCY AT FROG NEUROMUSCULAR JUNCTION IN LOW CALCIUM SALINE SOLUTIONS. NARITA, K., KITA, H. and YASUGI, E. Dept. of Physiology, Kawasaki Medical School, Kurashiki, Okayama 701-01

The effects of hypertonicity on the increase in miniature end-plate potential (MEPP) frequency that occurs during tetanic stimulation (50 Hz, 2-3 min) of the motor nerve have been investigated in Ca^{2+}-free, Mg^{2+}EGTA-containing (0Ca-MgEGTA) solutions. The change in $[Ca^{2+}]$ in the nerve terminal ($[Ca^{2+}]_i$) was estimated from quantal release frequency by using Barton et al.'s model (1983). Adding 25, 50, 75 and 100 mM sucrose to the 0Ca-MgEGTA solution elevated the basal $[Ca^{2+}]_i$ 1.4-, 1.8-, 2.5- and 2.8-fold respectively, and enhanced the rate of increase in $[Ca^{2+}]_i$ during the tetanus 1.9-, 3.0-, 4.6- and 5.0-fold respectively. Concanavalin A (con A) suppressed the elevated MEPP frequencies produced by hypertonic solutions to almost the basal level in isotonic solutions. Despite of the depressive action, tetanic stimulation in hypertonic solutions containing con A produced comparable increases in MEPP frequencies to those obtained in hypertonic solutions without con A. The addition of 2 or 3 mM caffeine to the 0Ca-MgEGTA solution elevated the MEPP frequency to the level attained in the solution containing 25 or 50 mM sucrose. However, the tetanus in the caffeine solutions did not cause such increases as those obtained in the hypertonic solutions.

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MINIATURE SYNAPTIC POTENTIALS RECORDED FROM CEREBELLAR PURKINJE CELL DENDRITES IN VITRO. OKAMOTO,K., KIMURA,H. and SAKAI,Y. Dept. of Pharmacology, National Defense Medical College, Tokorozawa, Saitama 359

Spontaneous potentials distinct from spontaneous Na- or Ca-spikes were intracellularly recorded from the distal and proximal regions of the dendrite of Purkinje cells in guinea pig cerebellar slices. Their amplitude histograms indicated that they were composed of unitary potentials with an amplitude of 0.4 or 0.5mV. Statistical analyses on the amplitude histogram, using Poisson and binomial distributions, suggested their quantal nature and multi-synaptic origins. The potentials were in a negative direction at a resting or less negative potential levels and inverted to be positive at more negative potential levels, and were abolished by amino acid antagonists such as bicuculline, picrotoxin and strychnine and by low external Cl^- concentrations, indicating their inhibitory nature. The potentials were also blocked by high Mg and tetrodotoxin, implying the synaptic origin. These findings suggest that the spontaneous activities of cerebellar interneurons such as stellate and basket cells are reflected by these spontaneous miniature synaptic potentials.

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ANTIDROMIC REGULATION OF THE SYNAPTIC TRANSMISSION IN THE BULLFROG SYMPATHETIC GANGLIA. TOSAKA, T., TASAKA, J., KOBAYASHI, H. and MIYAZAKI, T. Department of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo 160

The fast excitatory postsynaptic potential (epsp) or the spike (weakly curarized) in the 10th paravertebral sympathetic ganglion of the bullfrog was recorded by the air-gap method. By applying the antidromic stimulation (20 Hz, for 15 sec) supramaximal for activating both B and C fibers, the amplitudes of the fast epsp or the spike decreased to 50 ± 10.7% (n=46) of the control and returned gradually to the original level 50 to 60 min later. In some preparations, a subsequent increase in amplitude over the control level following the phase of the decrement lasted for 2 hours or more. The antidromic activation of only B fibers contributed to lesser extent to the decrement. Reduction of external Ca concentration to 0.45 mM evidently antagonized the decrement, suggesting a release of some kind of transmitter may be involved in it. Phentolamine and phenoxybenzamine (both 10 μM) and naloxone (3 μM) were ineffective in antagonizing the decrement. Orthodromically induced repetitive activation of postganglionic C neurons failed to affect the spike amplitude of the B neurons. Posttetanic potentiation (PTP) was antagonized completely by antidromic stimulation of both B and C fibers, or partially by B fibers only. We therefore conclude that some fibers, mostly C, ascending in the sciatic nerve suppress the nicotinic synaptic transmission and PTP as well.

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SPIKELESS INTERACTION OF MOTONEURON DENDRITES IN CRAYFISH. TAKAHATA, M. and HISADA, M. Zool. Inst., Fac. Sci., Hokkaido Univ., Sapporo 060

Interaction not resorting to spikes between motoneuron dendrites was demonstrated by intracellular current injection in crayfish. Subthreshold depolarization of a motoneuron (Add MN) innervating the adductor exopodite, one of the closer muscles of uropod, increased the spike activity of the other motoneuron (Red MN No.1) innervating the reductor ex. which was synergistic to the Add. Hyperpolarization of the Add MN decreased Red MN's spike activity. This spikeless interaction was also observed among opener MNs but was confined to among synergistic MNs. Effective regions of current injection into the Add MN for changing Red MN's activity were limited to the dendritic branches distal (200-300μm) to the spike initiating site. The amplitude of EPSPs evoked in the Red MN by antidromic spikes of the Add MN was decreased by depolarization and increased by hyperpolarization. This showed that the interaction was mediated chemically. The connection seemed to be monosynaptic since EPSPs followed the repetitive stimulation of up to 200Hz. We concluded that the crayfish MN dendrites function not only as the input site but as the complex integrative element with the graded output to other MNs and, possibly, to various types of interneurons.

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SPECIALIZED STRUCTURE OF SCHWANN PROCESS IN THE EXCESS NERVE TERMINALS OF SOLEUS MUSCLE UMENO,K.,KAWASAKI,T. Dept. of Physiol., Fac. of Med., Toyama Med. Pharm. Univ., Toyama

In order to examine the manner in which excess terminals were eliminated from the end plate during polyneuronal innervation, light(a silver impregnation) and electron microscopic experiments were carried out on the soleus muscle of neonatal mice.

In light microscope the size(μ^2) of excess terminals in 10d and 16d after birth was 23±4 and 30±10, respectively. On the other hand the size of terminal portion observed out of the end plate in 10d and 16d after birth was 3.4±1.2 and 3.0±1.0, respectively.

In electron microscope electron-dense lamellated membrane whorl extending from the Schwann process indented the excess terminal with a slender axon(0.6±0.04,μ). Drastic invaginations and lamellated membrane whorls of the terminal's plasma membrane occurred at the site where the sheet of specialized Schwann process contact with the terminal's plasma membrane. Some times such Schwann process was filled with vacuolae(400-1500 A°). In addition finger like Schwann processes deeply indented the terminal or separated the terminal from the basement membrane. Thus lamellated membrane structures of Schwann process were encoutered especially in 2 out of 18 end plates(5d), in 22 out of 45 end plates(10d) and 8 out of 32 end plates(15d).

These findings suggested that the specialized Schwann cell may have involved in a reduction in the size of excess terminals in the period of polyneuronal innervation.

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CHEMOSENSITIVITIES OF BULLFROG SPINAL GANGLION CELLS. MORITA,K.,KATAYAMA, Y.,AKASU,T.* AND KOKETSU,K.* Med. Res. Inst., Tokyo Med. and Dent. Univ. Tokyo, and *Dept. of Physiol., Kurume Univ. Sch. of Med., Kurume.

Intracellular recordings were made from primary afferent neurons in vitro to examine their sensitivity to biogenic substances, GABA, acetylcholine(ACh), 5-HT, ATP, histamine and substance P. GABA-induced response was the same as previously reported. ACh caused a fast transient and slow depolarization, subsequently or separately; the former was nicotinic and the latter was muscarinic in nature. 5-HT also produced two kinds of depolarizations as ACh did. The fast transient one induced by ACh and 5-HT was due to increasing Na^+- and K^+-conductance, whereas the slow one was due to decreasing K^+-conductance. ATP(100 nM-1 mM) caused a slow hyperpolarization at relatively low concentrations and produced a slow depolarization at high concentrations by increasing and decreasing K^+-conductance, respectively. Histamine and substance P produced slow depolarizations by a decrease in K^+-conductance. The responses produced by these biogenic substances strongly interacted with each other due to their effects on resting membrane properties and/or receptor-ionic channel complex.

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EFFECT OF ACUPUNCTURE NEEDLING ON TETANIZED OR PERIVERTEBRAL MUSCLE IN THE RECOVERY PROCESS FROM REDUCED TWITCH OF GASTROCNEMIUS MUSCLE AFTER TETANIC STIMULATION.

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Contraction induced by electric stimulation of both sides of the guinea pig gastrocnemius muscle was recorded simultaneously in situ under nembutal anesthesia. Tetanic stimulation(10 Hz supermaximal), applied to the gastrocnemius muscle for 60 minutes, extremely reduced the height of isometric twitch contraction. This muscle state was defined as muscle stiffness, which causes local muscle pain. The insertion of a needle to a) the tetanized muscle or b) the perivertebral muscle facilitated the recovery from reduced twitch height after tetanic stimulation. The acupuncture effect on the tetanized muscle disappeared in the denervated muscle, while that in the perivertebral muscle disappeared after the sciatic nerve was severed. Acupuncturing the peri L4, L5, L6 or S1 caused similar effect on recovery of reduced twitch height after tetanus. Intravenous application of atropine(0.5 mg) abolished perivertebral acupuncture effect. From these results, it was concluded that the facilitatory effect of acupuncture might be induced by facilitated recovery of blood circulation. This facilitation of circulation in acupuncture on tetanized muscle might be caused by axon reflex and on perivertebral muscle by the somatoautonomic reflex.

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DORSAL HORN NEURONS RESPONSIVE TO SYMPATHETIC AFFERFNT INPUT FROM THE HEART IN THE CAT.

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Neurons responsive to electrical stimulation of the inferior cardiac nerve within the dorsal horn of the spinal cord (T2-T4) were studied in the cat anesthetized with urethane-chloralose. They were found within laminae I, IIo and IV-VI. All of them had a cutaneous receptive field in the left upper forelimb, axilla or chest wall. Their responses to cutaneous receptive field indicated that they were either nociceptive specific or wide dynamic range neurons. Namely, all of them were cutaneous nocineurons. The results first of all indicated that excitation of neurons within the spinal dorsal horn by cardiac sympathetic afferent may elicit pain since cutaneous nociceptive neurons are associated with pain. Secondly, convergence of cutaneous and cardiac sympathetic afferent inputs onto spinal dorsal horn neurons as revealed by the present study supports the convergence projection theory of the referred viscereal pain. Thirdly, wide dynamic range neurons responsive to cardiac sympathetic afferents may account for the origin of allodynia during the intermittent period of anginal attacks.

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EFFECTS OF TRIGEMINAL NERVE STIMULATION OF THE SPINAL ACCESSORY MOTONEURONS OF CATS.

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Synaptic potentials in the spinal accessory(Acc) motoneurons were recorded on stimulation of the trigeminal nerve branches, spinal trigeminal nucleus(SpN), and main sensory nucleus(VS). EPSP's with a latency of 3.0-8.8 msec(5.4±1.8 msec, N=7) and IPSP's with a latency of 4.0-9.8 msec(6.5±1.9 msec, N=20) were elicited in the trapezius(Trap) motoneurons by stimulation of the infraorbital(IO) nerve. In the sternocleidomastoid (SCM) motoneurons, IO stimulation evoked EPSP's with a latency of 3.0-8.3 msec(5.1±1.6 msec, N=19) and IPSP's with a latency of 4.0-9.0 msec(6.5±1.6 msec, N=16). Stimulation of the lingual and ophthalmic nerve branches also induced EPSP's and IPSP's in both Trap and SCM neurons with similar ranges of the latencies to those evoked by IO stimulation. While stimulation of SpN produced exclusively EPSP's with the latency range of 0.5-1.2 msec(0.7±0.2 msec, N=27) in Acc neurons, VS stimulation elicited not only EPSP's with a latency of 1.3-2.5 msec(1.7±0.3 msec, N=24), but IPSP's with a latency of 3.0-5.5 msec(4.1±0.8 msec, N=12) in these motoneurons. Since synaptic activation of Acc neurons from the primary trigeminal afferents were polysynaptic IPSP's as well as polysynaptic EPSP's, the present findings are strongly indicative of a presence of the projection pathway via VS rather than SpN in the trigemino-accessory reflex system.

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EXCITATORY INPUT FROM THE SKIN TO THE GASTROCNEMIUS MOTONEURONS IN THE LOCAL TETANUS CAT. KANDA, K. Dept of Physiology, School of Medicine, Chiba University, Chiba 280

Tetanus toxin (100-150 mouse LMD/kg of body weight) was injected into the medial gastrocnemius (MG) muscle. The acute experiment was performed during the period when the inhibitory synapses on the motoneuron were blocked by the toxin, but the excitatory group Ia synapses remained intact, that is, two days after the toxin injection. The cat was anesthetized with sodium pentobarbital. The maximum homonymous group Ia EPSPs and the responses to stimulation of the ipsilateral sural nerve (Sur PSP) were recorded from the MG motoneurons by using a glass capillary electrode filled with 4 M K-acetate. The latency of Sur PSPs measured from arrival of the villey in the fastest conducting afferents was 2.6 ± 0.4 ms (mean ± S.D.,range 1.8-3.3, n=55). The threshold for Sur PSP was about 1.5 to 2.0 times threshold for the most excitable fibers in the sural nerve. The Sur PSPs appeared to grow in accordance with the N_2 wave of the cord dorsum potential. These characteristics of Sur PSP in the present experiments were consistent with the previously reported results from the normal cats. The Sur PSPs of all motoneurons recorded in 8 out of 9 cats were purely depolarizing.These PSPs integrated over the initial 20 ms were directly correlated with the maximum amplitude of the homonymous Ia EPSPs (r=0.70, P 0.001). This suggests that the synaptic organization of both group Ia afferents and interneurons related to the Sur PSP are similar in nature.

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THE DEVELOPMENT OF THE STRETCH REFLEX IN THE RAT. ARASAKI, K. and KUDO, N. Dept. of Physiol., Inst. of Basic Medical Sciences, Univ. of Tsukuba, Ibaraki 305

The development of stretch reflex pathways was studied *in vitro* in foetal and newborn rats. The spinal cord below the mid-thoracic level was isolated with the hindlimb and its innervation kept intact. The preparation was bathed in Krebs solution (25±1°C) boubled with 95% O_2 and 5% CO_2. Intracellular recording from triceps surae (TS) motoneurones revealed that the excitatory postsynaptic potentials (EPSPs) were evoked by phasic stretch of the TS muscle in the newborn rat. The interval between the arrival of incoming volleys in the dorsal root and the onset of the EPSPs was about 10 msec, comparable to the latency of monosynaptic EPSPs evoked by electrical stimulation of the dorsal root. The reflex response could also be recorded electromyographically from the TS muscle when the muscle was stretched or the sciatic nerve was stimulated electrically. The reflex was found to be elicited by both the two procedures in all preparations of the newborn rat and the 20-day-old foetus (E 20, one day before birth), in about half of preparations at E 19, and in none at E 18. By contrast, natural and electrical stimulation of the plantar skin produced the reflex activity with longer latency in the flexor muscle (biceps femoris) in E 18 and older preparations. It is concluded that monosynaptic stretch reflex pathways start to function in the 19-day-old foetus, later than the skin evoked polysynaptic reflex.

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FORE- AND HINDLIMB COORDINATION DURING LOCOMOTION OF THE CAT. KATO,M., MURAKAMI,S. and HIRAYAMA,H., Dept. of Physiology, Hokkaido University School of Medicine, Sapporo, 060.

It was proposed that the ascending propriospinal tract system plays an important role for the coordination of fore- and hindlimbs during locomotion (Miller et al., 1973). If this assumption is acceptable, then in a preparation of which the interlimb reflex system was severed by bilateral hemisections at different levels of thoracic cord, the coordination must be greatly disturbed while in a preparation with one hemisection at thoracic level and contralateral hemisection at cervical level, thus preserving at least interlimb reflex system in one side, the coordination should be reasonably retained. With this argument in mind we have studied fore- and hindlimb coordination during locomotion on the following 4 groups of chronic cats; 1) cats with unilateral hemisection at Th_{12}, 2) cats with serial hemisection at Th_{12} and contralateral Th_5, 3) cats with unilateral hemisection at C_2 and 4) cats with serial bilateral hemisections at C_2 and contralateral Th_5. Coordination between fore- and hindlimbs were equally severely distorted in both Th-Th bilaterally hemisected cats and C-Th bilaterally hemisected cats. From these findings it was suggested that interlimb reflex systems play little role for coordination of the for- and hindlimbs during locomotion.

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IDENTIFICATION OF RENSHAW CELLS IN THE DECEREBRATE CATS MAINTAINING REFLEX STANDING POSTURE.
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In the decerebrate cats maintaining reflex standing posture, it is possible to identify alpha-MNs innervating hindlimb extensor muscles by stimulating nerve endings with wire electrodes implanted into the muscles. In this study, using the same method for activation, attempts have been made to identify Renshaw cells belonging to the same groups of alpha-MNs and to study how the firing rate of Renshaw cells changes with the level of postural muscle tone in the hindlimb. To record the discharge of Renshaw cells, glass micropipettes (6-8 MΩ) were used. 8 Renshaw cells belonging to the soleus were identified extracellularly in L7 and S1 segments by their characteristic discharge patterns. During reflex standing of the animal, Renshaw cells discharged tonically with firing rate ranging 8-70 spikes/s. The higher was the level of postural muscle tone, the higher was the firing rate of Renshaw cells. When postural muscle tone of the hindlimb was reduced to several levels by stimulating the dorsal part of the mid-pontine tegmentum (DTF), spontaneous firing rate and total number of discharges elicited by test antidromic shock reduced significantly during DTF stimulation. All these results suggest that Renshaw cells participate in the regulation of postural muscle tone receiving inputs both from alpha-MNs and from supra-spinal structures such as DTF.

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PHENOMENOLOGICAL MODEL FOR SPINAL MECHANISMS OF STEPPING.
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The spinal rhythm generator for stepping of a single limb was simulated by a dynamical system with a set of nonlinear differential equations. The system took a form similar to the half-center organization, consisting of extensor and flexor motor nuclei with mutual inhibition. The set of equations could be reduced to that of cusp catastrophe, described by a single state variable (representing a difference of activities of the two motor nuclei) and two control parameters (corresponding to two pre-motor nuclei, each exerting reciprocal innervation and coactivation of the motor nuclei). Further dynamics in the cusp surface was determined by introducing linear dynamic equations for the control parameters with feedback from the state variable. Then the system could be made unstable by additional inputs (corresponding to a command) to the control parameters and various oscillations were evoked. In one condition a sudden transition took place from extension to flexion and the reverse transition occurred gradually. In another, sudden changes occurred at both transitions. By changing interaction coefficients or the additional inputs to the control parameters, the cycle period of oscillation could be varied in a similar manner to experimental observations where the duration of extensor activity mainly determined the cycle period.

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THE EXTEROCEPTIVE VIBRATION-INDUCED TOE FLEXION REFLEX.
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A tonic finger flexion reflex was induced by mechanical vibration of the finger or palm skin, and similarly vibration applied to the sole skin occured the toe flexion reflex. This toe flexion reflex extremely reduced by local anaesthesia of the skin, suggesting that this reflex is derived from the skin rapidly adapting mechanoreceptors.

On the instruction to maintain a voluntary tension of the big toe, a plot of vibration-induced tension against the voluntary tension was found to increase linearly at the voluntary tension 0～500g and to be approximately steady at the voluntary tension 500～1000g. Also, this reflex was facilitated with upward rotation of the ankle joint (90～70°) and induced tension further increased with background weak voluntary tension of the toe. These results suggest that background voluntary tension and upward rotation of the ankle joint give facilitative effects to this reflex. Besides, cutaneous acupuncture inhibited this reflex.

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FACILITATION AND INHIBITION OF INFRAORBITAL NERVE-ELICITED JAW OPENING REFLEX OF THE RAT INDUCED BY CUTANEOUS STIMULATION. Y.YAMAGUCHI, A. NISHIGORI, W.YASUNO AND M. KAWAMOTO Laboratory of Clinical Physiology, College of Biomedical Technology, OSAKA University.

Effects of cutaneous stimulation (CS)on infraorbital nerve-elicited jaw opening reflex (JOR) were examined in rats anesthetized with pentobarbital sodium. The reflex EMG's were recorded from the digastric muscles. The CS was single pulse electric one and was given through needles inserted into the skin of the respective face, fore-or hind-paw. The reflex EMG's of JOR consisted of R1(latency;5-7 msec) and R2 (latency; 10-15 msec) responses. The facilitation followed by inhibition of the JOR was induced by the CS. The facilitation appeared from 0 to 30 msec for R1 and from -5 to 20 msec for R2. The inhibition followed and lasted for about 300 to 500 msec. Facilitation or inhibition induced by the CS could be modified by a preceding CS.
The facilitation and inhibition observed in infraorbital nerve-elicited JORs were more marked than those in pulp induced ones.

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REFLEX RESPONSES OF BILATERAL DIGASTRIC MUSCLES DURING MASTICATION. MORIMOTO, T., INOUE, T., SAKAN, I. and KAWAMURA, Y. Dept. Oral Physiol., Osaka University, Dental School, Kita-ku, Osaka 530.

The modulation of reflex responses in the bilateral digastric muscles during mastication was analysed in the rabbit. Single electrical shocks were applied unilaterally to the inferior alveolar nerve at different times of the masticatory stroke. Reflex responses in these two muscles were, more or less, facilitated in the jaw closing phase and depressed in the jaw opening phase. The facilitatory effect during the jaw closing phase was stronger in the muscle of the stimulated side than that of the unstimulated side. These digastric responses were also modulated depending on whether the animal masticated on the stimulated side or on the unstimulated side. However, the amplitudes of bilateral digastric responses were significantly correlated with each other irrespective of the masticatory side. Furthermore, the correlation coefficient was greater during mastication than when the animal was in the rest.
These results suggest that there is a neuronal mechanism which ensures coordination between bilateral digastic muscles to the unexpected sensory input during mastication.

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CORRELATION OF DISCHARGE PATTERNS OF BULBAR RETICULAR NEURONS WITH JAW MUSCLE ACTIVITY AND JAW MOVEMENT DURING FOOD INGESTION IN CATS. HIRABA, K., SAHARA, Y., TAIRA, M. and NAKAMURA, Y. Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Bunkyo-ku, Tokyo 113

Single unit activities were recorded in the bulbar reticular formation during food ingestion in painlessly head-restrained, unanesthetized cats. Forty neurons modulated the firing in the same rhythm as the masticatory jaw movement; 34 of them showed a burst during each jaw-opening phase (the period from the jaw-closed position to the maximum jaw-opening position). By averaging EMG of jaw-closing and -opening muscles by spikes of these neurons, 4 were found to be inhibitory neurons projecting to jaw closer motoneurons (IM) and other 4 to be excitatory neurons to jaw opener motoneurons (ED). A positive correlation was demonstrated between the instantaneous firing frequency (IFF) of these units and the velocity of jaw-opening movement in 2 of 3 IM and 2 of 3 ED tested. IFF also showed a positive correlation with the jaw displacement from the jaw-closed position in 2 of the 3 IM and one of the 2 ED. In this ED and one of the 2 IM, the spike number composing a burst showed a positive correlation with the duration of the jaw-opening phase and/or the maximum jaw displacement; the maximum firing frequency positively correlated with the maximum jaw-opening velocity and displacement. Thus, IM and ED would control the velocity and displacement of jaw-opening during mastication by modulating the firing frequency as well as the number of spikes in each burst.

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NATURE OF CORTICALLY INDUCED RHYTHMICAL MASTICATORY ACTIVITY OF HYPOGLOSSAL MOTONEURONS IN CATS. SAHARA, Y., KATOH, M. and NAKAMURA, Y. Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Bunkyo-ku, Tokyo 113

Intracellular recordings were made from 12 and 13 hypoglossal motoneurons (XII MNs), identified by antidromic spikes evoked by stimulation of the medial and lateral branches of XII nerve, respectively, in immobilized encephale isolé cats, with the following results. (1) Repetitive stimulation of the orbital gyrus (OG) induced a rhythmical depolarizing potential (DP) with spikes in these XII MNs. Between successive DPs, the potential either returned to the same level as before stimulation or moved to a slightly hyperpolarized level. Short-train pulses applied to the OG evoked either EPSPs or EPSP-IPSPs, but not IPSPs alone. (2) The rhythmical DP was superimposed by increased synaptic activation noise. Intracellular de- and hyperpolarizing currents depressed and enhanced the DP amplitude, respectively. (3) The hyperpolarizing potential between successive DPs never reversed to a DP by intracellular Cl^- injection, even after the cortically evoked IPSPs changed into DPs. (4) The cortically induced rhythmical burst in XII nerve persisted after i.v. injection of strychnine (0.1 mg/kg).We conclude that the cortically induced masticatory activity in XII MNs is due to their rhythmical excitation, and strychnine-sensitive inhibition is not essential for the central generation of the masticatory rhythm. No evidence was obtained for an inhibition between excitatory phases.

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AMYGDALOID AND CORTICAL CONTROL OF MASSETERIC MOTONEURONS IN THE RAT. OHTA, M. Dept. of Physiology, Fac. of Dentistry, Kyushu University, Fukuoka 812

The author's group clarified that electrical stimulation of the frontal cortex (FCtx) or the central amygdaloid (CAm) nucleus induced jaw movements primarily due to activation of jaw opening motoneurons. In the present study, the effects of electrical stimulation of the FCtx or the CAm nucleus were examined on the masseteric motoneurons. Thirty six % of them were inhibited and 11 % were facilitated from both FCtx and CAm nucleus. Facilitation or inhibition from either one summated on that from another. Eleven % were inhibited from either one and 5 % were facilitated exclusively from the FCtx although the rest (about 36 %) were unaffected. Facilitation was demonstrated to be due to relatively long latency EPSPs and inhibition was due to short latency IPSPs and concomitant reduction of membrane resistance. In conclusion, the FCtx and the CAm nucleus have convergent control of jaw movements primarily due to activation of jaw opening motoneurons and inhibition of jaw closing ones.

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AMBIGUUS MOTONEURONS FOR PRODUCING ULTRASOUND VOCALIZATION IDENTIFIED BY ANTIDROMIC STIMULATION OF THE SUPERIOR AND THE INFEFIOR LARYNGEAL NERVES. YAJIMA, Y. and HAYASHI, Y. Dept. of Physiol. Hyogo College of Med., Nishinomiya, Hyogo 663

A total of 61 neurons in the nucleus ambiguus(NA) were activated antidromically to stimulation of the inferior laryngeal nerve (ILN) with mean peak latency of 2.9 ±0.6 msec. In most of NA neurons rhythmic bursts were found either spontaneously or in response to stimulation of the dorsal part of the central gray matter (PVG). Antidromic spikes to ILN stimulation collided with these bursts,either spontaneously occurring or evoked by PVG stimulation. Forty five NA neurons were activated antidromically by stimulation of the superior laryngeal nerve (SLN) with mean peak latency of 2.1 ±0.8 msec. Most of them had neither background discharges nor bursty response to PVG stimulation. Histological examination revealed that most NA neurons activated antidromically by ILN stimulation were found in or quite close to the dorsal formation of the ventral division of NA, while those driven by antidromic SLN stimulation were in the ventral formation of the same division of NA.

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FORELIMB STEPPING MECHANISMS OF THE BRAINSTEM IN THALAMIC CATS. SHIMAMURA, M., FUWA, T. and KOGURE, I.* Dept. of Neurophysiol., Tokyo Metropol. Instit. for Neurosci., 2-6 Musashidai, Fuchu-city, Tokyo 183

In the present experiments, we attempt to identify supraspinal mechanisms which play a role of forelimb stepping in thalamic cats on a treadmill. 1. Thalamic cats exhibited forelimb stepping on a moving belt, even after transection of the low spinal segment (T10). However, high spinal (C1) and an additional of the low spinal (T10) transected cats did not obtaine forelimb stepping, even after administration of nialamide and l-DOPA. Whereas hindlimb stepping was evoked by those drugs. 2. Bursting discharges were observed the paralemniscal part of the nucl. reticularis pontis oralis (NRPO) while the forelimb stepped on the moving belt. Those discharges appeared earlyer than the flexor EMGs. 3. Repetitive stimulation applied to the NRPO, induced the forward movement of the ipsilateral forelimb, and the backward movement of the contralateral one. 4. The lesion was made the NRPO, reduced the forelimb stepping on bothside.

Our results suggeste that the NRPO play an important role in the initiation of the forelimb stepping in thalamic cat.

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THE CHANGES IN THE MEMBRANE RESISTANCE OF SOLEUS MOTONEURONS BY STIMULATION OF DORSAL PART OF CAUDAL TEGMENTAL FIELD (DTF) OF THE PONS. T. SAKAMOTO, Y. ATSUTA* AND S. MORI Dept. of Physiol., Asahikawa Med. Coll., Asahikawa ; Dept. of Orthoped., Asahikawa Med. Coll., Asahikawa*

In the acute precollicular-postmammillary decerebrate cat maintaining reflex standing posture, DTF stimulation (rectangular pulses 0.2 ms, 50 pulses/s, 10-30 uA, 10 s) elicits long-lasting hyperpolarization in the range between 4 to 14 mV of extensor alpha-motoneurons in the hindlimbs. To understand the neuronal mechanism for sustained increase of membrane potential, the changes in the membrane input resistance was studied. In 10 motoneurons with resting membrane potential from -52 to -65 mV, hyperpolarizing current pulse (2-15 uA, 20 ms duration) was injected through the impaling microelectrode every 0.5 s before, during and after DTF stimulation. The input resistance of them decreased by 18.2 $\pm$ 6.4 (from 7.2 to 44) % during DTF stimulation (n=8). After termination of stimulation, input resistance returned to the prestimulus level. In two motoneurons, input resistance of them increased during DTF stimulation, and further increase of it was observed after termination of stimulation. These results suggest that the long-lasting hyperpolarization of extensor alpna-motoneurons is elicited, at least in part, by active postsynaptic inhibition. The long-lasting hyperpolarization may be also accompanied by the suppression of excitatory input.

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DISCHARGES OF NEURONS IN THE DORSAL PART OF THE MIDPONTINE TEGMENTUM DURING LOCOMOTION. KAWAHARA, K., OHTA, Y., MORI, S. AND * KANAYA, T. Dept. of Physiol., Asahikawa Med. Col. Asahikawa; * Dept. of Otolaryngol., Asahikawa Med. Col., Asahikawa 078-11

In the dorsal part of the midpontine tegmentum (DTF) of locomotor preparations, there are neurons synaptically drived by stimulation of the mesencephalic locomotor region(MLR). In this investigation, the discharge properties of these neurons in the DTF area(DTF neurons) projecting down the lumbosacral spinal cord are analysed during locomotion and are compared with those of the laterally located(L,R 0.5-2.0) reticulospinal neurons(RS neurons). Almost all the DTF neurons showed a spontaneous discharge with a frequency of about 10 spikes/s during upright standing. All the RS neurons identified did not possess spontaneous activity. The mean conduction velocities of axons of the DTF and the RS neurons were 70 and 130 m/s, respectively. During MLR elicited locomotion, the discharge rate of the DTF neurons increased tonically. The RS neurons showed a rhythmic discharge when the animal started to walk. The cyclic modulation of the discharges was in phase with the locomotor cycle, with a maximal discharge rate at the swing phase of the ipsilateral hindlimb. These results suggest that the DTF and the RS neurons may play different functions in control of locomotion. The DTF and the RS neurons may participate in the tonic regulation of postural muscle tone and in the phasic regulation of the phase of the step during locomotion, respectively.

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CHARACTERISTIC DISCHARGES OF HINDLIMB MUSCLES DURING ALTERNATING LIMB LOADING IN A REFLEX STANDING DECEREBRATE CAT. Y. OTA, K. KAWAHARA AND S. MORI Dept. of Physiol., Asahikawa Med. Coll., Asahikawa

The acute precollicular-postmammillary decerebrate cat maintains reflex standing due to decerebrate rigidity. Stimulation to the dorsal portion of the cuneiform nucleus (mesencephalic locomotor region) elicits regular alternating limb loading between the hindlimbs (still stepping). Regular still stepping was recorded as oscillation in hindlimb forces with a mean period about 0.5 s and with a mean amplitude of 8 to 14 % of total body weight. The time between the beginning of increase and the peak of the force was less than 0.2 s. During a single stepping of hindlimbs, distal muscles to the knee joint such as tibialis anterior (TA) and gastro-soleus (GS) muscles exhibited reciprocal discharges. The beginning of TA bursting discharge (mean duration ; 0.06 s) preceeded GS bursting discharge (mean duration ; 0.1 s). The beginning of GS discharge preceeded about 0.05 s to the increase in the force. Proximal muscles to the knee joint, irrespective of flexor and extensor muscles, exhibited in phase bursting discharge (duration 0.05 to 0.15 s). The timing of bursting discharge of these muscles was identical with that of GS muscle. The sequence of activation of hindlimb muscles during still stepping was different from that of locomotion and that of scratching. These results seem to disclose the third type of rhythm generating capability within the spinal cord.

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ACTIVITY OF DEITERS' NEURONS DURING CONTROLLED LOCOMOTION IN THE MESENCEPHALIC CAT. T. KANAYA, K. KAWAHARA* AND S. MORI* Dept. of Otolaryngol., Asahikawa Med. Coll., Asahikawa ; Dept. of Physiol., Asahikawa Med. Coll., Asahikawa*

In acute mesencephalic cat, stimulation of mesencephalic locomotor region (MLR) can elicit "controlled locomotion" on a moving treadmill. During locomotion, we analysed discharge characteristics of Deiters' neurons in relation to step cycle. To do this, treadmill speed was varied keeping the stimulus intensity to MLR constant. Deiters' neurons were identified antidromically by stimulating L2 segment of the spinal cord and recorded extracellularly. Deiters' neurons were divided into three groups depending on discharge pattern associated with hindlimb movement. The first group of neurons (7/12) exhibited almost tonic discharge when step cycle was over about 1 s. With shortening of step cycle, these neurons exhibited bursting discharge at the beginning of stance phase. The second group neurons (3/12) exhibited bursting discharge at contralateral stance phase when step cycle was over about 1 s. With shortening of step cycle, these neurons tended to discharge twice in a single step cycle, one at the beginning of ipsilateral stance phase and the other at the beginning of contralateral stance phase. The third group of neurons (2/12) exhibited tonic discharge with no relation to duration of step cycle. All these results suggest that the discharge of Deiters' neurons are modulated in a several ways depending on the step cycle and movement of ipsi and contralateral hindlimb.

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REFLEX RESPONSES OF SINGLE INFERIOR SALIVATORY NEURONS TO STIMULATION OF TRIGEMINAL NERVE AND CHORDA TYMPANI IN THE CAT. ISHIZUKA, K., MURAKAMI, T., YOSHIHARA, M. and UCHIYAMA, M. Dept. of Oral Physiol., Nippon Dental Univ., Niigata, Niigata

Responses of 57 single inferior salivatory neurons,identified by antidromic spikes evoked by stimulation of the tympanic nerve,were tested to stimulation of the ipsilateral infraorbital(IO),inferior alveolar(IA),lingual(LN) and chorda tympani(CT) in the urethan-chrolarose anesthetized cat.Thirty five neurons polysynaptically responded with spike potentials to stimulation of one or more these nerves(R neurons) ,while the remaining 23 neurons did not respond to stimulation of any of them(NR neurons).We did not find any difference between R and NR neurons in their conduction velocities.Convergence of excitatory input from some stimulated nerves was found in the majority of R neurons.Reflex spike responses appeared with a latency of 4.0-15.7 msec to IO stimulation,4.6-13.2 msec to LN stimulation,3.6-21.0 msec to IA stimulation and 6.4-19.0 msec to CT stimulation.These reflex properties were similar to those of superior salivatory neurons previously reported.The reflex activities to LN stimulation were evoked by excitatory input from the trigeminal afferent,but not from the taste afferent.

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NEURONS RESPONSIVE TO STIMULATION OF NEUROMA DEVELOPED IN THE INTRA-ORBITAL NERVE WITHIN THE CAUDAL MEDULLA OBLONGATA IN THE CAT.
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Experiments were carried out in adult cats. The intraorbital nerve was severed on one side. The transected central stump was ligated in order to prevent its regeneration back into the peripheral receptive field. Two weeks later, the trigeminal subnucleus caudalis and its nearby bulbar lateral reticular formation were explored with a glass microelectrode filled with pontamine sky blue in 1M Na acetate under urethane-chloralose anesthesia. Neurons responsive to gentle tapping over the neuroma were found either within the marginal as well as substantia gelatinosa layers of the trigeminal subnucleus caudalis, or within the lateral part of subnucleus reticularis dorsalis, or within the dorsolateral part of subnucleus reticularis ventralis. These area are known to contain trigeminal nociceptive neurons by previous studies in our laboratory. Neurons responsive to tapping over the neuroma were not found within the magnocellular layer of the trigeminal subnucleus caudalis, but this region showed an alteration in the sensory map.

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MORPHOLOGICAL AND ELECTRICAL PROPERTIES OF RED NUCLEUS NEURON USING THREE DIMENSIONAL RECONSTRUCTION BY COMPUTER.
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HRP stained Red Nucleus neurons by intracellular injection were reconstructed using three dimensional image processing system. Eight neurons were reconstructed with resolution of 0.2μm. Average surface area of soma-dendritic membrane of cells was 140,000 μm^2 and ratio of soma area to the total surface area was 0.11. The sum of the 3/2 power of the dendritic diameters decreased with distance from the soma. Rall's 3/2 power law was not applicable to the Red Nucleus neuron. From the data of dendritic diameters, we caliculated input impedance of Red Nucleus neurons. Total imput impedance ranged from 3.3 to 17.6 MOhm and the average was 9.4 MOhm (Ri: 100 OhmCM, Rm: 5000 OhmCM^2). Average ratio of soma impedance to the total dendritic impedance was 2.9.

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Appearance of new component in the cortico-rubral EPSPs after the establishment of classical conditioning in the cat.
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We examined whether sprouting and formation of new synapses could be a neuronal basis of learning. Classical conditioning of forelimb flexion mediated by the red nucleus (RN) in the cat occurred after pairing stimulation of the cortico-rubral fibers with forelimb shock for 7-10 days. We investigated the cortico-rubral EPSPs by intracellular recording from the RN neurons in the conditioned and control cats. After conditioning, a new fast-rising component appeared superimposed on the normal slow-rising cotico-rubral EPSPs. Time-to-peak of these EPSPs in the conditioned cats is significantly shorter ($p<0.005$) than that of the control cats. The most likely interpretation is that the cortico-rubral fibers sprouted and formed new functional synapses on the more proximal portion of the soma-dendritic membrane of RN neurons. Other posibilities were also discussed.

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CEREBRAL AND CEREBELLAR PROJECTIONS TO THE PARVOCELLULAR PART OF THE RED NUCLEUS IN CATS. OKA, H. Department of Physiology, Fukui Medical School, Fukui 910-11

Climbing fiber responses in the cerebellar cortex to stimulation of the parietal association cortex were indicated to be mediated mostly through the rubro-olivary pathway originating in the parvocellular part of the red nucleus (RN), and climbing fiber responses to stimulation of the frontal motor cortex were also suggested to be conveyed to some extent in this way. In the present study, the parvocellular part of the RN was identified in the midbrain by recording evoked responses to stimulation of the parietal association cortex, and HRP was injected electrophoretically in this part through recording microelectrodes. Localization of cerebral and cerebellar projection neurons was then examined anatomically by utilizing retrograde axonal transport of HRP. Labeled cells with HRP were distributed largely in area 5 and moderately in area 6 of the cerebral cortex ipsilateral to the HRP-injected side. Labeled cells were also located predominantly in the lateral nucleus and slightly in the interpositus nucleus of the contralateral cerebellum.

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ELECTROPHYSIOLOGICAL STUDIES ON THE MODE OF CEREBRO-OLIVARY CONNECTIONS AND THEIR RESPONSIVENESS. SHIMONO, T. and KANG, Y. Dept. of Physiol., Fac. of Med., Kyoto University, Konoe, Yoshida,Sakyo-ku, Kyoto 606

Mode of cerebral projections to the inferior olive (IO) and its responsiveness were studied by recording IO unitary and field potentials evoked by stimulation of the cerebral cortex (area 4, 5 and 6) and the pyramidal tract at the level of the trapezium (Py) and the pyramidal decussation (PX) in nembutalized cats. Most of the neurons in the medial accessory olive received inputs from area 4, 5 and 6, mainly via extra-pyramidal pathways. Most of the neurons in the medial part of the principal olive (PO) accepted also cortical inputs from area 4, 5 and 6, but mainly through the cortico-olivary fibers in the pyramidal tract. The majority of the neurons in the medial part of the dorsal accessory olive (DAO) received cortical projections only from area 4, mainly via axon collaterals of the cortico-spinal tract fibers (CST). The excitatory transmission via axon collaterals of CST was confirmed by amplitude diminution of EPSP in the collision test with combined stimulation of Py and PX. Responsiveness of DAO and PO neurons was examined by repetitive stimulation (1-100 Hz) of area 4, Py or PX. The maximum amplitude of EPSP was attained at 20-50 Hz. However, the action potential was deteriorated at ca. 10 Hz, which was probably due to the unique pattern of the IO action potentials with prolonged depolarization and after-hyperpolarization.

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THE AXONAL TRAJECTORIES TO THE EXTRAOCULAR MOTONEURON POOLS OF INHIBITORY VESTIBULO-OCULAR NEURONS ACTIVATED FROM THE CAT ANTERIOR, POSTERIOR AND HORIZONTAL SEMICIRCULAR CANALS. UCHINO, Y. and SUZUKI, S. Dept. of Physiology, Kyorin University School of Medicine, Shinkawa 6-20-2 Mitaka-shi, Tokyo 181

The branching pattern of inhibitory vestibuloocular neurons and their synaptic contacts with extraocular motoneurons were studied by means of spike-triggered averaging and local stimulation techniques. Individual vestibuloocular neurons activated by stimulation of the ampullary nerve of the anterior semicircular canal inhibited motoneurons in both the ipsilateral (i-) trochlear nucleus and i-inferior rectus motoneuron pools. Individual vestibuloocular neurons receiving input from the ampullary nerve of the posterior semicircular canal inhibited motoneurons in both the i-inferior oblique and i-superior rectus motoneuron pools. Probably, these axonal trajectories underlie conjugate eye movement during vertical head rotation.
No conclusive evidence was found to indicate that single inhibitory vestibular neurons receiving input from the horizontal semicircular canal give off axon collaterals to the i-abducens and the contralateral medial rectus motoneurons. Separate projection of HCN-related neurons to motoneurons supplying the lateral and medial rectus muscles might be useful for convergence during horizontal head movement.

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INTRACELLULAR STUDIES OF SUPERIOR COLLICULUS NEURONS PROJECTING TO THE TECTORECIPIENT ZONE OF THE LATERAL POSTERIOR NUCLEUS IN CATS. TAKIMORI, T., TAKAHASHI, Y. AND OGAWA, T. Dept. of Physiol., Akita Univ. Sch. Med. 1-1-1 Hondo, Akita 010.

To elucidate what types of superior colliculus (SC) neurons (morphologically identified) send their axons to the tectorecipient zone of the lateral posterior nucleus (LP) and which type(s), W and/or Y, of retinotectal fibers are connected to these SC neurons, intracellular recording and Procion yellow or Lucifer yellow dye injection were performed in Nembutal-anesthetized cats. The type of retinal afferents to a paticular SC neuron was identified by assessement of conduction velocity from a latency shift for EPSPs evoked by stimulation of optic chiasm (OX) and that of optic tract (OT), and the distance of OX-OT. The projection of this neuron to the LP was established by antidromic activation resulting from stimulation of LP ipsilateral to the SC studied. Four SC neurons were successfully identified: three were of the wide-field vertical type and one of the narrow-field vertical type. They were all W-recipient neurons. In the present experiments, we have not demonstrated whether Y-recipient SC neurons project to the LP as well.

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BEHAVIORAL MODIFICATION OF VISUAL RESPONSES OF NEURONS IN THE MONKEY SUBSTANTIA NIGRA. HIKOSAKA, O. and WURTZ, R.H.* Dept. of Physiol., Toho Univ. Sch. Med., Ota-ku, Tokyo 143 and Lab. of Sensorimotor Research, National Eye Institute, N.I.H., Bethesda, MD. 20205, U.S.A.*

Visual responses of substantia nigra pars reticulata (SNr) neurons were studied in the alert monkey (Macaca mulatta). Visual stimuli were presented on a tangent screen while the monkey was fixating on a small center spot. Nearly a half of neurons in the lateral part of the SNr decreased their high background discharge rate following onset of visual stimuli. They had relatively large receptive fields centered on a visual hemifield contralateral to the recording site. Small light spots were more effective than larger stimuli; no preference to stimulus movement or orientation was observed. These visual responses were enhanced under either of the following conditions: (1) when the monkey made a saccadic eye movement to the stimulus immediately after its onset, (2) when the monkey kept fixating following brief presentation of the stimulus and after a few sec made a saccade to the remembered stimulus position, (3) when no light spot was present for the monkey to fixate before the visual stimulus appeared. These results suggest that different types of cognitive processes such as attention, short-term memory and anticipation modulate visual signals in SNr neurons which are then used for initiation of movements, particularly saccadic eye movements.

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PLASTIC CHANGES IN PROJECTION FROM LOCUS COERULEUS (LC) TO LATERAL GENICULATE NUCLEUS (LGN) FOLLOWING ABLATION OF VISUAL CORTEX (VC) IN ADULT RATS. SAKAGUCHI, T., SHIROKAWA, T. and NAKAMURA, S. Dept. of Neurophysiology, Inst. of Higher Nervous Activity, Osaka University Medical School, Kita-ku, Osaka 530

Stenevi et al. showed histochemically that unilateral ablation of VC resulted in an increase of noradrenaline-containing terminals (NA-terminals) in LGN in adult rats. Electrophysiological studies were made to see if the increased NA-terminals in LGN were due to an increase in the projection from LC. In rats with VC of one side ablated the projection from LC to LGN was found increased on the same side. LC neurons innervating LGN and frontal cortex (FC) simultaneously were encountered more frequently in VC ablated rats than in control, this making a significant contribution to the increase of the LC-originated, LGN-destined projection after ablation of VC. Findings were obtained suggesting that many of the LC neurons innervating FC and VC in the preoperative stage came to send axon collaterals to LGN after ablation of VC (pruning effect), so that they were identified as projecting to FC and LGN simultaneously.

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UNIT ACTIVITY OF THE PONTINE NUCLEUS NEURON IN BEHAVING MONKEYS.
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Discharge patterns of neurons in the pontine nucleus were studied in association with forelimb movement, while monkeys were performing a visual tracking task. After the monkeys had learned the task, an Evarts' type cylinder was fixed to the skull on the occipital bone. The cylinder was tilted so that roentgenography was used to fix the cylinder at a correct position over the pontine nucleus. The dura was replaced with a thin artificial dura. This manoeuvre reduced much troubles caused by thickning of the dura. Thin and long tungsten electrodes (5-10MΩ) were used to record unit activity. Ninety nine pontine units were obtained from three monkeys. Of these 99 units, 24 were related to flexion at the wrist joint, 27, to extention, 7 to both extention and flexion, 27 to holding a handle and the remaining 8 were related to holding as well as movement of the handle. Another six were pause units. Additional 48 units were obtained in more superficial areas, but their characteristics were very similar to those of pontine nucleus neurons.

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RHYTHMIC BURST ACTIVITY OF THE PONS IN EXPERIMENTAL TREMOR IN MONKEYS.
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Possible descending pathways mediating tremor have been studied in monkeys with experimental spontaneous tremor produced by a mesencephalic ventromedial tegmental lesion. Based on the previous findings on the spinal cord, the reticulospinal tract has been highly suspected as a candidate. Therefore, pontine and medullary regions were systematically explored in two monkeys. Ventral or horizontal stereotactic trackings were made under X-ray control. Tremor time-locked rhythmic activity was found mostly in the ipsilateral dorsal tegmental area both in pons and medulla. It was aligned rostrocaudally along the aqueduct and IVth ventricle, about 2mm lateral to the midline. This region corresponds to the N. tegmentalis pontis oralis and caudalis, which are thought to be the origin of pontine reticulospinal tract. Among several natural peripheral stimulations tried, the compression of the tremor muscle induced the same spike discharge. And the stimulation of this recorded point in the pons evoked localized contraction of the trembling muscles.

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EXISTENCE OF CALCIUM SPIKE IN THE SUPRAOPTIC NEURON OF RAT HYPOTHALAMIC SLICE.
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We investigated the existence of calcium spike in supraoptic (SON) neurons using rat hypothalamic slices by intracellular recording. Replacement of sodium by choline or application of TTX in a normal medium lead to a decrease in the rate of rise and an increase in the duration of the action potentials which were elicited by depolarizing current injection. The action potentials were abolished by addition of EGTA or cobalt in the TTX containing medium. The amplitude of the action potentials depended on $[Ca^{2+}]_o$ in the low sodium medium. A ten-fold increase in $[Ca^{2+}]_o$ caused about 29 mV increase in the action potentials. When TEA was added to the low sodium medium, the duration of the action potential was prolonged up to 400 msec - 1sec. Addition of TEA in the medium after calcium channel blockade by cobalt made only a little increase in the duration of the action potentials. The calcium spike was observed in nine out of ten SON neurons tested. As it has been reported that interneurons were not many in the rat SON, most of all tested neurons may be neurosecretory neurons. We concluded that neurosecretory neurons in the rat SON could generate calcium spikes.

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POSSIBLIE ROLE OF PARAVENTRICULAR AND SUPRAOPTIC NUCLEI ON AUTONOMIC FUNCTION.
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Possible projections from regions of paraventricular (PVN) and supraoptic (SON) nuclei to the spinal cord were investigated in cats. Electrical stimulation of PVN and SON regions (3-5 pulses at 200 Hz, 0.5 msec duration, 10-50 μA) evoked distinct preganglionic sympathetic nerve discharges recorded from white ramus (T_2, T_3) after latency of 40-70 msec. Stimulation of other areas in the hypothalamus with this intensity, however, produced none or weak responses with longer latency. Recording from neurons in the PVN and SON area following stimulation of the intermediolateral column (ILC) of spinal cord (T_2, T_3) revealed that some neurons were antidromically activated by such stimulus. Nine neurons out of 294 responsed to the cord stimulation with the constant latency and were able to follow high frequency stimulating pulses, and thus were thought to be antidromically activated by such stimulus. Two out of these 9 neurons successfully tested were also antidromically activated by stimulation of the pituitary stalk. Our results show that there are a few number of neurons in or near the PVN, projecting directly to the ILC of the thoracic cord and that they contribute to the autonomic function.

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CONNECTIONS BETWEEN DORSOMEDIAL MEDULLA NEURONS AND THE PARAVENTRICULAR NUCLEUS AND THEIR POSSIBLE INVOLVEMENT IN THE CONTROL OF CARDIOVASCULAR SYSTEM.
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The paraventricular nucleus (PVN) of the hypothalamus has been suggested to be involved in the control of the autonomic nervous system. The present experiments were designed to examine effects of electrical stimulation of the PVN and of activation of the carotid baroreceptors on firing activity of neurons in the dorsomedial medulla which participate in the cardiovascular regulation. In urethane-anesthetized male rats, extracellular recordings were made from 607 neurons within the nucleus tractus solitarius and its vicinity. Twenty one percent of the neurons were orthodromically excited, 6% were inhibited and 3% were antidromically activated following PVN area stimulation. Antidromic spike latencies were 22-64 msec. Pressure pulse stimulation of the isolated carotid sinus produced excitation in 7 and inhibition in 13 of the 81 tested neurons which orthodromically responded to the PVN area stimulation. Of the 8 tested neurons which were antidromically activated, one neuron was excited and another neuron inhibited by the pressure pulse stimulation. These results provide electrophysiological evidence for reciprocal connections between dorsomedial medulla neurons and the PVN, and give a support to the hypothesis that the PVN is involved in the neural control of cardiovascular system.

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CONTROL OF GASTRIC ACID SECRETION THROUGH PARAVENTRICULAR NUCLEUS.
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Secretion of gastric acid (GAS) in response to peripheral and/or central administration of chemical or electrical stimuli can be differentiated by vagotomy. GAS has been shown to be controlled by specific lateral hypothalamic (LHA) neurons. Insulin or 2-DG applied to the LHA by microinjection or iontophoresis has experimentally induced GAS. The paraventricular nucleus (PVN) has now been found to also affect GAS. GAS was produced more copiously and more quickly by rostral PVN lesion than by lesion of the ventromedial- (VMH) or dorsomedial- (DMH) nucleus, and nearly as much by caudal PVN lesion. Microinjection of 2-DG was more potent in reversing the effects of PVN lesion than in reversing the effects of VMH or DMH lesion, or in intact animals. Electrical stimulation in the PVN suppressed both LHA activity and GAS.

The PVN may be an additional site from which GAS is centrally affected. Whether control is due to PVN neurons, or fibers of passage must be determined by further electrophysiological investigation.

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ELECTROPHYSIOLOGICAL STUDIES OF THE DEVELOPMENT OF SUPRACHIASMATIC NEURONAL ACTIVITY IN HYPOTHALAMIC SLICE PREPARATIONS. SHIBATA, S., UEKI, S. and OOMURA, Y.* Dept. Pharmacol., Fac. Pharmaceut. Sci.,*Dept. Physiol., Fac. Med., Kyushu Univ., Fukuoka.

The autoradiographic 2-DG method indicated circadian rhythm of glucose utilization in the rat suprachiasmatic nucleus (SCN) on 1st day. However, no electrophysiological study has documented postnatal development of rat SCN neurons. We therefore examined the rate and pattern of neural discharge in the SCN during early development. The firing rate of the SCN neurons was low on the 7th (3.2 ± 2.3 Hz) and 11th (3.4 ± 2.4) days, and increased dramatically by the 14th day (5.2 ± 2.8) and reached the adult rate (6.3 ± 3.1). The discharge rate of neurons in the ventrolateral part of the SCN (VL-SCN) was higher than that of dorsomedial SCN (DM-SCN) neurons in every developmental stage. Activity characteristics of the DM-SCN neurons during development were unaffected by bilateral enucleation on the 3rd day and by housing in continuous darkness, while activity in the VL-SCN decreased. Thus neither light illumination nor intact eyes seem to be essential for the development of the DM-SCN neurons.

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NEURONS PROJECTING FROM THE NORADRENERGIC A1 REGION TO THE PREOPTIC AND ANTERIOR HYPOTHALAMIC AREA. KABA, H., SAITO, H., OTSUKA, K., SETO, K. and KAWAKAMI, M.* Dept. of Physiology, Kochi Medical School, Nankoku, Kochi and *Dept. of Physiology, Yokohama City University School of Medicine, Yokohama

At least two types of neurons ('slow' and 'fast' cells) projecting from the ventrolateral medulla oblongata to the preoptic and anterior hypothalamic area were antidromically identified in urethane-anesthetized female rats. Slow cells had longer antidromic spike latencies (mean ± s.e.m., 45.5 ± 1.4 ms). By contrast, fast cells had shorter antidromic spike latencies (7.3 ± 0.5 ms). The antidromic excitation of slow cells was followed by an inhibitory period of spontaneous firing. The occurrence of both antidromic spikes and post-stimulus inhibition of the majority of these neurons was completely abolished by the injection of 6-hydroxydopamine (6-OHDA) but not 5,7-dihydroxytryptamine directly into the medial forebrain bundle. The post-stimulus inhibition was also blocked by picrotoxin administrated intravenously at the dose of 4 mg/kg. These results suggest that the 6-OHDA-sensitve slow cells are most likely A1 noradrenergic neurons and their post-stimulus inhibition is mediated via the activation of both their axon collaterals and GABAergic neurons. We investigated the effects of estrogen on the strength of the post-stimulus inhibition of slow cells. We now report that estrogen reduces the potency of the inhibition.

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EFFECT OF INTRACEREBROVENTRICULAR ADMINISTRATION OF 2-DG ON LHA NEURONAL ACTIVITY IN UNANESTHETIZED RATS. KATAFUCHI, T., YOSHIMATSU, H. and OOMURA, Y. Dept. of Physiology, Fac. of Medicine, Kyushu University 60, Fukuoka 812

Long term unit recordings were obtained from neurons of the lateral hypothalamic area (LHA) in unanesthetized rats. 2-deoxy-D-glucose (2-DG) (15.2 μmole/10 μl/10min) was injected by cannula into the III ventricle. Eleven of 36 neurons exhibited a marked increase in activity for several hours after 2-DG injection, which was followed by decrease in about half of them (5/11). Thirteen neurons displayed a decrease in activity. Feeding behavior occurred in about 80% of all cases during these changes in neuronal activity. The reciprocal relationship between activity of two neurons, recorded simultaneously, which responded oppositely to 2-DG was shown as a negative peak in cross correlation functions. These results, and our previous experiments demonstrating an increase in efferent discharge rate of adrenal nerve by electrical stimulation in the LHA (not published), suggest that the LHA neurons are involved in feeding behavior and hyperglycemia induced by 2-DG.

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GLUCOSE RESPONSIVE NEURONS IN THE RAT HYPOTHALAMIC DORSOMEDIAL NUCLEUS, IN VITRO.
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The dorsomedial nucleus (DMH) is considered to be important in body weight regulation. The DMH connects anatomically to the ventromedial nucleus (satiety center, VMH) and lateral area (feeding center, LHA) of the hypothalamus, which contain glucoreceptor and glucose-sensitive neurons, respectively. We studied effects of glucose on DMH neurons, using rat hypothalamic tissue slices, and examined the structure of the glucose responsive neurons in the DMH, using intracellular horseradish peroxidase (HRP) injection. Results were: (1) Responses to glucose (5.5-20mM) were divided into excitation (E), excitation followed by inhibition (E-I) and inhibition (I). (2) Of 212 DMH neurons tested in 45 slices, 69 (32.5%) responded to glucose (E-type, 36/212(17.0%); E-I type, 18/212(8.5%); I type, 15/212(7.1%)). (3) Some DMH neurons responded to glucose even when the DMH was isolated from the VMH and the LHA. (4) Glucose responsive neurons identified by intracelluler HRP injection (n=6) had fusiform or bipolar cell bodies and richly branching main dendrites. (5) Non glucose responsive neurons (n=14) also had fusiform or bipolar cell bodies but the main dendrites tended to be straight and run parallel.

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BEHAVIORAL SIGNIFICANCE OF MONKEY LATERAL HYPOTHALAMIC CHEMO-SENSITIVE NEURONS.
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To elucidate the behavioral significance of the monkey lateral hypothalamic (LHA) chemo-sensitive neurons, the effects of iontophoretic application of glucose, noradrenaline (NA), dopamine (DA) and satiety and hunger substance candidates discovered in the rat, 2-deoxy tetronate (2-DTA) and 3-deoxy pentonate (3-DPA) on the monkey LHA neurons and their feeding-related neuronal activities were investigated. Glucose, NA and 2-DTA decreased in discharge rate in about 25 %, 50 % and 15 % of LHA neurons respectively, while DA increased or decreased in about 40 %. 3-DPA facilitated in about 15 %. Discharge rates of these chemo-sensitive cells changed more often during eating/reward period than those of insensitive cells. Furthermore, many glucose- and 2-DTA-sensitive cells decreased in activity during bar press/food procurement period. The results suggest that the chemo-sensitive neurons are involved in the reward mechanism of feeding behavior, and glucose-sensitive neurons which include 2-DTA sensitive cells play a specific role in hunger motivated food procurement behavior.

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HETEROGENOUS PROJECTIONS FROM AMYGDALA TO HYPOTHALAMUS SHOWN BY RETROGRADE HRP.
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Amygdaloid afferents to the lateral (LHA) and ventromedial (VMH) hypothalamic nuclei were studied by retrograde transport of horseradish peroxidase (HRP) following microiontophoretic injection into various subdivisions of these areas. Results were : 1) Anterior regions of the LHA predominantly receive afferents from the anterior part of the medial nucleus (AM), and afferents from the VMH. Posterior regions of the LHA receive more afferents from the central nucleus (AC) than from the AM. Although afferents from the AC predominate, lateral parts of the LHA diffusely receive strong afferents from the whole amygdala complex. 2) The VMH receives strong projections from the AM. Dorsal regions of the VMH predominantly receive afferents from the anterior part of the AM, and the posterior part of AM projects to ventral regions. The data reveal topographic differences in the amygdaloid projections to the hypothalamus which may, in part, reflect functional roles of the AM and AC in control of feeding, sex, and defense behavior.

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THE PATHWAY WHICH MEDIATES THE SLOWEST WAVE OF FORNIX EVOKED POTENTIAL IN THE MAMMILLARY BODY OF CAT.
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The fornix evoked potential was recorded in the mammillary body (MB) of cat anesthetized with Nembutal. The potential consisted of three components, named wave 1, 2 and 3. My previous study including local coagulation or small knife cut within the diencephalon showed that the wave 2 was evoked via the descending column of fornix. In the present experiments, the pathway through which the wave 3, the slowest component of the potential was evoked was studied. Ipsilateral fornix body was stimulated to evoke the wave 3. Bilatral frontal cut was done between the septum and the descending column by the use of a small thin knife so as to interrupt the precommissural fibers of fornix. But the cut did not affect the amplitude of wave 3. This suggested that the precommissural fibers did not mediate the wave. Hypothalamic stimulation just ventromedial to the descending column evoked a wave similar to wave 3 in the MB. The wave 3 might be evoked via a tract analogous to the medial corticohypothalamic tract which exsists in rats and monkeys, since the stimulation site corresponds to the tract.

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SOME CHARACTERISTICS OF VESTIBULO-SYMPATHETIC RESPONSES.
T. MIYAZAWA, T. ISHIKAWA. Dept. of Physiology, Nihon Univ. Sch. Med., Itabashi, Tokyo 173

Chloralose-urethane anesthetized cats were used as experimental animals. Electrical stimulation to the vestibular nerve (V), two times the threshold value of the nystagmus, with a 1-msec duration was applied and a stimulus to the lumbar nerve (L) of 0.3-msec duration and 30 uA in strength was given. The potential responses in the sympathetic renal nerve (RN) and the somatic lumbar nerve were recorded simultaneously. The interval between the lumbar nerve-conditioning stimulus and vestibular nerve-testing stimulus was changed and the recovery curve was drawn. The recovery curves for the test response from the somatic nerve and from the sympathetic nerve were different. After cervical dorsal part transection, the recording response from the intermediolateral column of C3 and the sympathetic renal response were still present on vestibular stimulation, but the somatic response had disappeared. Based on these results, the effects of vestibular nerve stimulation on the sympathetic and somatic reaction are assumed to have different actions in relation to the spinal cord, so that their characteristics are not the same.

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INTERFERENCES IN VISCERAL AFFERENT INPUTS AND SOMATIC SIGNALS INDUCED BY ELECTRIC STIMULATION FOR BODY SURFACE.
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Single neural unit discharges have been recorded extracellularly in the ventrobasal complex (VB) in the thalamus of the immobilized cat with multi-micro electrodes technique and their positions have been determined histologically.

16 of 141 units responded to not only somatic stimuli, but also heart beats. These receptive fields were distributed in whole body.

Applying electric stimulation to the receptive fields of these neurons, the interference phenomenon such as the inhibition of the heart beat synchronized discharges were observed.

The positions of these neurons were located in whole VB and the localization was not recognized. It is suggested that the convergences of the afferent inputs of somatic and visceral sensation were participated with the higher central nervous system.

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VAGUS-RELATED NTS NEURONS PROJECTING DIRECTLY TO THE FOREBRAIN IN RATS. NOSAKA, S. Department of Physiology, Mie University School of Medicine, Edobashi, Tsu 514

Electrical stimulation of the hypothalamus in chloralose-urethane anesthetized rats evoked antidromic responses in about 5 % of those neurons in the nucleus tractus solitarius (NTS) which responded orthodromically to the vagus nerve stimulation. The NTS neurons with such a direct forebrain projection (F-NTS neurons) were distributed mostly in the lateral part of the ipsilateral commissural NTS. Latencies of the antidromic responses ranged from 20 to 75 ms, indicating that the axons of the F-NTS neurons were unmyelinated. Orthodromic responses (latencies, 20 to 80 ms) were observed in 6 out of 23 F-NTS neurons to the same stimuli that evoked the antidromic responses. Sites that elicited antidromic responses in the NTS neurons upon electrical stimulation covered almost all medial hypothalamic nuclei, but 65 % of them were localized in the preoptic-anterior region. All orthodromic responses to activation of the vagus afferents (either myelinated or unmyelinated) were polysynaptic in nature. Three F-NTS neurons were found to project to different hypothalamic regions by axonal branching. In conclusion, vagal visceral input is transmitted polysynaptically to the F-NTS neurons and then, conveyed to the forebrain via the direct pathway. A role in a feedback control mechanism of the forebrain activities is also speculated as another possible function of this pathway.

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AUTONOMIC MODULATION IN MONKEY PREFRONTAL NEURONAL ACTIVITY DURING FEEDING BEHAVIOR. AOU, S., OOMURA, Y., NISHINO, H., INOUE, M. and NAKANO, Y. Lab. of Humoral Control, Nat. Inst. Physiol. Sci., Okazaki 444; Dept. of Physiol., Fac. Med., Kyushu Univ., Fukuoka 812; Dept. of Physiol., Toyama Med. and Pharmaceu. Univ., Toyama 930-01.

To elucidate the role of subcortical autonomic system in feeding-related neuronal activity in the monkey prefrontal cortex, effects of i) iontophoretic application of noradrenalin (NA) and acetylcholine (ACh), ii) microinjection of glutamate into the locus coeruleus (LC) and the substantia innominata (SI), iii) electrical stimulation of the lateral hypothalamic area (LHA), and iv) intravenous injection of glucose, on prefrontal neuronal activity during a bar press feeding task were investigated. Iontophoretic application of NA and chemical stimulation of the LC decreased in discharge rates of the same prefrontal neurons. Iontophoretic application of ACh and chemical stimulation of the SI facilitated firing rates. The majority of cells which showed a decrease in activity during the bar press period responded to NA and electrical stimulation of the LHA. Furthermore, this activity change was diminished by intravenous injection of glucose. There was no apparent relationship between the sensitivity to ACh and the firing pattern during the feeding task. These findings suggest that the LC-NA system in the prefrontal cortex plays an important role in the glucose related neural regulation of bar press/food procurement behavior.

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ODOR RESPONSES OF MEDIODORSAL THALAMIC NUCLEUS (MD) NEURONS IN THE RABBIT. IMAMURA, K. AND ONODA, N. Dept. of Physiol. Gunma University School of Medicine, Maebashi, Gunma 371

In order to study the olfactory pathway through the thalamus, single unit responses of the MD to odors and to electrical stimulation of the lateral olfactory tract (LOT) and that of the olfactory projection area in the neocortex (OPA), were examined in the light anesthetized rabbit. Animal products, dry food pellets, 8 pure chemicals were used as odor stimuli with artifitial air intake cycles. Seventeen relay neurons which showed both transsynaptic responses to LOT shocks and antidromic responses to OPA shocks were located in the medial segment of the MD. The population of relay neurons responded to odors of animal products and/or dry food pellets exclusively was low, when compared with odor response charateristics of OPA neurons (Proc. Japan Acad. 57:300, 1980). These results indicate that olfactory inputs terminate in the OPA via the MD and that response selectivity to biologically significant odors would appear through the thalamocortical pathway.

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MORPHOLOGICAL INVESTIGATION OF THE VA-VL NEURONS OF THE CAT THALAMUS BY INTRACELLULAR HRP STAINING.

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Morphological as well as electrophysiological investigation of the VA-VL neurons of the cat thalamus was performed by intracellular HRP staining on stimulation of the entopeduncular(Ent), cerebellar(CN), caudate(Cd) nuclei and motor cortex. Fourty-five neurons were stained successfully by intracellular HRP injection. They were classified into two groups, i.e., Ent-responding(n=20) and CN-responding ones(n=25). The size of somata and the dendritic arborization of them were compared with those of the thalamocortical(T-C) relay neurons labeled retrogradely by injection of HRP into area 6. Most of neurons stained intracellularly have radially projecting dendrites as T-C neurons have, and the size of somata and dendritic radius did not differ significantly except few cases, which showed different dendritic pattern, i.e., dendrites spread obliquely. None of them could be identified as projection neurons electrophysiologically, so they assumed to be interneurons. In several neurons, axons were also stained and they could be traced in the thalamus. Most of CN-responding neurons send axons dorsolaterally whereas those receiving Ent-IPSPs send axons mostly ventrolaterally in the cases activated antidromically by Cd stimulation.

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NECK AND VESTIBULAR INPUTS TO CCN NEURONES AND PATTERNS OF THEIR CONVERGENCE IN THE CAT. Hirai, N., Hongo, T., Sasaki, S., Yamashita, M. and Yoshida, K. Dept. of Physiol. Institute of Basic Medical Sciences, Univ. of Tsukuba, Ibaraki 305

Neurones of the central cervical nucleus (CCN), the origin of a spinocerebellar tract, were found to receive excitation from neck muscle and vestibular afferents. The effect from the neck was produced via mono- and/or oligosynaptic pathways, and originated from dorsal neck muscles. The excitation was strongest from the afferents coming into the same segments, but nerves entering more caudal segments could also be effective. Each CCN neurone received input from restricted muscles, often from a single muscle. At C2-C3, for example, many neurones were excited from either splenius(Spl) or biventer cervicis-complexus (BCC), and convergence from both was rare. Intraaxonal staining of group Ia muscle afferents with HRP showed that they terminate in CCN, suggesting that the muscle spindle afferents were responsible for the excitation of CCN neurones. CCN neurones were also excited from the contralateral labyrinth, either from the anterior or posterior but rarely from the horizontal canal. Neurones excited from the posterior canal were activated from Spl and its synergists, and those excited from the anterior canal were activated from BCC. It is concluded that the convergence from neck and vestibular inputs onto individual CCN neurones occurs in a highly specific manner.

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DISCHARGES FROM PURKINJE CELLS AND INTRACELLULAR POTENTIALS IN DEITERS' NEURONES RESPONDING TO MECHANICAL PERTURBATIONS DURING LOCOMOTION. UDO, M.[1] and MATSUKAWA, K.[2] [1]Div. Neuromuscular Skills, Fac. Health and Sport Sci., Osaka Univ., Toyonaka, Osaka 560, and [2]Dept. Cardiac Physiol., Nat. Cardiovascular Center Res. Inst., Suita 565

During locomotion of cats decerebraed at the precollicular and premammillary level, single unit activity from Purkinje cells (P-cells) and intracellular recording from Deiters' neurones (D-neurones) were performed, while mechanical perturbations (taps of 50-550 gr-wt) were applied at the paw dorsum of the left forelimb (LF). In response to the LF taps during stance phase of the LF, the ongoing LF stance phase was shortened and the ongoing swing phase of the right forelimb (RF) was shortened correspondingly. This maintained the bisupport phase in which both forelimbs were in stance. A notable response to this LF tap was earlier onset of the RF extensor activity than in unperturbed steps. D-neurones controlling the RF extensors and P-cells connected to these D-neurones were identified by their antidromic and orthodromic activations (Udo et al. Exp. Brain Res. 41, 1981). In response to the LF tap, a major part of the P-cells (18 out of 28) showed a significant depression in their spike discharges and the D-neurones showed depolarizing potentials. These responses of P-cells and D-neurones occurred in a close time relation to the earlier onset of the RF extensor activity, suggesting the cerebellar contribution to interlimb coordination during locomotion.

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CONVERGENT INPUTS FROM THE INTERPOSITUS AND THE DENTATE NUCLEI TO SINGLE CORTICOFUGAL NEURONS IN THE MOTOR CORTEX. YAMAZAKI,M., FUTAMI,T. SHINODA,Y. Dept. of Physiology, Sch. of Medicine, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo.

Cerebellar projections to the motor cortex were analyzed stimulating the interpositus(IP) and the dentate(DN) nuclei separately and recording intracellualr potentials from single corticofugal neurons in the motor cortex and thalamocortical neurons(TCN) in VL in anesthetized cats. Fast PTNs received disynaptic and slow PTNs di- or tri-synaptic EPSPs fro the cerebellum. Most of them received convergent inputs from both IP and DN and at least a portion of this convergence occurred at the level of VL. Morphology of TCNs in VL and cerebellar nucleus neurons(CBN) was examined, using intracellular injection of HRP. TCNs had radiating dendrites with a large number of spines in all directions(dendritic fields;300-500um) and they projected to a very wide area in the motor cortex with multiple branches. Terminal boutons were distributed mainly in layer III and V, sparsely in layer I,II and VI. Single CBN axons branched and arborized diffusely in VL and terminal boutons were grouped in a grape-like fashin.

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VOLUNTARY LIMB MOVEMENT IN DIFFERENT CONTEXTS AND CEREBELLAR PURKINJE CELL ACTIVITY OF THE MONKEY. MANO,N., KANAZAWA,I. and YAMAMOTO,K. Dept. of Neurophysiol.,Tokyo Metropol. Inst. for Neurosciences, Fuchu-shi, Tokyo 183

Single Purkinje cell (P-cell) activity was analyzed in relation to both visually guided tracking movement and returning movement after executing the tracking. In the former movement, the monkey had to flex or extend the wrist about 30° from a neutral position, tracking a target's displacement, precisely controlling the timing and velocity of wrist movement to recieve a reward. In the latter, the monkey was not required to return the wrist in a specified fashion, the returning being irrelevant to a reward, but only to begin a next trial. Firing probability of complex spikes (CSs) of about 100 P-cells recorded from 4 rhesus monkeys phasically increased at the beginning of the tracking movement, coincidentally with rapid frequency modulation of simple spikes (SSs). But, the phasic increase of CS activity was totally absent or very weak and the frequency of SSs modulated gradually, when the monkey returned the wrist to starting neutral position, even though the direction, amplitude and peak velocity of wrist movement were same as those of the tracking movement. The average firing rate of CSs at maintained wrist position, 30° flexed or extended, was almost same as the rate at neutral position, suggesting the implausibility to explain the different CS activity in the two movements by the different initial wrist positions.

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EYE MOVEMENTS AREA IN THE CEREBELLAR FLOCCULUS OF PIGMENTED RABBITS DETERMINED BY MICROSTIMULATION MAPPING. NAGAO,S.,ITO,M. and KARACHOT,L. Dept. of Physiol., Fac.of Med., Univ. of Tokyo, Bunkyo-ku, Tokyo 113

Twelve mongrel pigmented rabbits were operated under anesthesia, and a platform for head fixation and holes for tracking were prepared. Triple-barreled microelectrodes were filled with Pontamine sky blue(dissolved in 0.5M $NaCH_3CO_2$), Fast green(in 2N NaCl) and Nigrosine(in water pH8-9). The left flocculus was tracked at 500μ intervals systematically. At each occasion when the microelectrode arrived at the Purkinje cell layer, trains of repeated pulses(300Hz,pulse width 0.2msec,duration 5sec,<30μA) were applied through the microelectrode. Three zones H,V and R were defined by the horizontal movement in the ipsilateral eye, vertical movement in the ipsilateral eye and rotatory movement in the contralateral eye respectively. Two V zones were identified in the rostral and cadal parts of flocculus, both of which extended dorso-ventrally. One H zone was located beteween the V zones. R zone was observed in the ventral folia, extending rostro-caudally. In addition, a small zone for blinking was found in the rostro-ventral extreme, and another zone for neck muscle contraction was located in the ventral flocculus.

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AXON BRANCHES OF VISUAL CLIMBING FIBERS PROJECTING TO DIFFERENT PARTS OF THE VESTIBULOCEREBELLUM. TAKEDA, T. and MAEKAWA, K. Department of Physiology, Jichi Medical School, Minamikawachi-machi, Tochigi-ken, 329-04 Japan.

The vestibulocerebellum receives visual signals via climbing fibers (cf), of which those to the flocculus originate from the dorsal cap of inferior olive. Using pigmented rabbits, we have investigated origin of visual cf to the nodulus and uvula and cf collateral projections between the flocculus and the nodulus or the uvula. Horseradish peroxidase injected into nodulus or uvula labelled neurons in the dorsal cap as well as those in the nucleus β. Electrical stimulation of nodulus or uvula evoked antidromic responses in the dorsal cap, which partially occuluded with both the antidromic response from the flocculus and the orthodromic response from the optic nerve. It also evoked cf responses in the floccular Purkinje cells (mean latency, 4.0 ms) which occluded with optic nerve evoked cf responses. They were due to axon reflex via cf bifurcation because they were not changed after lesion of the dorsal cap. Another combinations, flocculus (stimulation) -nodulus(recording) and uvula(stimulation) -flocculus(recording) also showed cf collateral projections. Above data evidenced that substantial proportion of dorsal cap neurons transmits visual signals both to the flocculus and to the nodulus or uvula simultaneously through cf collaterals.

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DEPRESSION OF CEREBELLAR PURKINJE CELL ACTIVITY DURING GUSTATORY STIMULATION OF THE FROG TONGUE. HANAMORI, T. and ISHIKO, N. Dept. of Physiol., Miyazaki Med. Coll., Miyazaki

Electrical stimulation of the bullfrog glossopharyngeal (IXth) nerve evoked negative field potentials of molecular layer origin in the bilateral cerebellar hemispher. It also elicited complex and simple spikes indicative of Purkinje cell excitation. Because these electrical activities were attributed to excitation of the IXth nerve afferents other than low threshold mechanosensitive fibers, it was considered that gustatory fibers of higher threshold may transmit afferent signals from the tongue to the cerebellum. Application of taste solution to the tongue was found difficult to detect apparent change in the spontaneous firing of simple and complex spikes so far recorded extracellulary. However, when Purkinje cells were activated electrically during taste stimulation, initiation of Purkinje cell spikes was depressed in 88 % of the samples studied. The depressant action depended on the quality of taste substaces, the order being found to be $CaCl_2$ > NaCl = water > sucrose > acetic acid > quinine.

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ANOMALOUS CEREBELLOFUGAL PROJECTIONS PRODUCED BY REGENERATED AXONS FOLLOWING TRANSECTION OF THE SUPERIOR CEREBELLAR PEDUNCLE IN KITTENS. KAWAGUCHI, S., MIYATA, H. and KATO, N. Dept. of Physiology, Inst. for Brain Res., Fac. of Med., Kyoto Univ., Kyoto 606

Previous report (Neurosci. Lett. 25 '81) described the occurrence of remarkable axonal regeneration of cerebellothalamic projection neurons in kittens following transection of the superior cerebellar peduncle. Terminals of the regenerated fibers, like those of intact cerebellofugal fibers, were most densely distributed contralaterally in the red nucleus and thalamic VA-VL nuclei. Besides such projections, the regenerated cerebellofugal fibers produced sparse anomalous projections. These projections can be classified into two categories according to the sites of terminations. One is the projections onto the interpeducular nucleus and the ventrolateral part of the thalamic LP and VPL nuclei which do not exist in intact animals. The other is the uncrossed ipsilateral projections onto the central tegmental field, red nucleus, periaqueductal gray, zona incerta, field of Forel, and thalamic CM, Pc, NCM, CL and VA-VL nuclei; completely crossed contralateral and recrossed ipsilateral projections onto the same structures exist in normal animals. The anomalous projections of the first category were much less than those of the second category. Thus, with respect to structures, the ability of growing axons to recognize the proper target during ontogenesis is well maintained during regeneration and anomalous projections do not appear a serious problem of axonal regeneration.

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AN ANALYSIS OF THE FUNCTIONAL CONNECTION BETWEEN THE MOTOR AND THE PARIETAL CORTEX OF THE CAT. KANG, Y., ENDO, K. and ARAKI, T. Dept. of Physiology, Faculty of Medicine, Kyoto University, Kyoto 606

Intracellular potentials in cortical neurons in areas 4γ and 5 of the cat were recorded following intracortical microstimulation (ICMS) in areas 5 and 4γ respectively. Some of PT and non PT cells in areas 4γ and 5 were antidromically activated by ICMS in areas 5 and 4γ respectively. Thus, two systems of reciprocal connections between areas 4γ and 5, by axon collaterals of PT cells and by cortico-cortical neurons, were found. A prepotential was usually observed in the rising phase of antidromic spike potentials in PT cells produced by ICMS but not by pyramid stimulation. This prepotential may be explained by assuming that the branching point of cortico-cortical collaterals from the parent axon of PT cells is very near the soma and that there is some conduction delay of cortico-cortical antidromic impulses at the branching point. No prepotential mentioned above was observed in antidromic spike potentials of cortico-cortical neurons. ICMS produced also PSPs in PT cells. EPSPs, showing remarkable frequency potentiation similar to recurrent EPSPs, were thought to be evoked by activation of cortico-cortical collaterals of PT axons. Those, showing different patterns of frequency potentiation, seemed to be evoked by activation of cortico-cortical neurons and of collaterals of cortico-nuclear neurons.

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Electrophysiological study on the corticoreticular projection neurons of the cat. JINNAI, K. Department of Physiology, Shiga University of Medical Sciences, Seta, Ohtsu, Shiga 520-21

Thirty-two motor cortical neurons which were antidromically activated by stimulation of the ipsilateral medullary reticular formation (RF), but not activated by stimulations of other medullary structures surrounding RF, were regarded as corticoreticular neurons. They consisted of slow PT neurons with collateral branch to RF (slow PT-RF neuron: N=22), fast PT neurons with collateral branch to RF (fast PT-RF neuron: N=6), and neurons which project on RF independently from the PT-system, i.e., RF(non-PT) neurons (N=4). On stimulation of the thalamus and the cerebellum, fast PT-RF neurons were strongly activated in the increasing phase of surface positive cortical evoked potentials, whereas slow PT-RF and RF(non-PT) neurons did not fire in this phase, but fired in the surface negative phase. It was concluded that the activities of the slow PT-RF and RF(non-PT) neurons are controlled mainly by excitatory thalamic inputs onto the distal and superficial part of their apical dendrites.

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PROJECTION FROM THE THALAMUS TO MOTOR EFFECT ZONE OF PREINSULARIS (2PR.I) OF THE SECOND SOMATIC SENSORY CORTEX. MORI, A., WATERS, R.S*., and ASANUMA, H*. Dept. of Physiol., Nihon Univ. Sch. of Med, Tokyo, and The Rockefeller Univ. New York, N.Y. USA

We have reported (Mori et al. Neuroci. Abstr. 7, 1981) that intercortical microstimulation (ICMS) in area 2 pr.i produced movement with very weak currents (10μa or less). In the present study, we examined projections from the thalamus to area 2 pr.i using HRP. A single microlectrode was inserted through a camber into area 2 pr.i. HRP was injected at the site where ICMS of a 2 pr.i neuron produced movement of the contralateral side of the body. The area which produced digit movements received input from neurons in the medial division of the ventroposterior lateral nucleus (VPLm), the ventroposterior medial nucleus (VPM), the medial division of the posterior group of nuclei (Pom) and the magnocellular medial nucleus (MC). The area which produced hindlimb movements received input from neurons in the VPL1, VPLm, VPM, Pom, the lateral division of the posterior group of nuclei (Pol) and MC. The no motor effect zone (forepaw) received input from VPLm. The results suggested that these area 2 pr.i neurons received input from VPL, VPM, Po and MC and those thalamocortical projections may play an important role in the execution of cortically induced movements.

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LOW THRESHOLD OF RAT SmI BARREL NEURONS IN RESPONSE TO WHISKER DEFLECTION.

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Spike potentials were recorded from neurones in the rat primary somatosensory cortex under urethane anaesthesia. Single whiskers were deflected with a controlled stimulator. Comparison was made between different cortical layers of the receptive field size (in terms of the number of whiskers connected to a neuron), stimulus threshold, tuning curve slope and sustained/transient aspect of the response. Neurons situated at the level of barrels (layer IV) differed from those above (layers II and III) or below (layer V) in having lower threshold and smaller receptive field (spatially sharp-tuned). More neurons were found in the layer IV that showed sustained discharges during whisker deflection and flat tuning curves, thus oriented for amplitude coding. By contrast, neurones in layers II, III and V tended to display transient responses and more steeply sloping tuning curves, suggesting a dependence on velocity component of stimulus.

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LOCALIZATION OF PULPAL-THALAMIC NEURONES SENDING THEIR AXONS TO SI (3a).

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Our previous studies revealed that the major cortical projection area from facial skin was located in the anterior part of coronal gyrus (3b), while at the bottom of coronal sulcus (3a) from tooth pulp and masseteric muscle.

In α-chroralose anesthetized cats, location of trigeminal thalamic neurones which sent their axons to 3a and 3b was studied by recording orthodromic potentials evoked by stimulation of facial skin, pulp and masseteric nerves and antidromic potentials activated by stimulation of 3a and 3b in which the maximum cortical responses from trigeminal inputs could be recorded. Distribution of thalamic neurones sending their axons to 3a and 3b was confirmed anatomically by electrophoretic injection of 2.5% HRP (WGA) into maximum trigeminal projection site in SI.

It was, therefore, assumed that the thalamic neurones with facial input sending their axons to 3b was located in the ventral part of rostral VPM, and those with pulpal and masseteric input were in the dorsal part of caudal VPM.

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TWO KINDS OF THETA-RELATED UNIT ACTIVITIES IN THE RABBIT HIPPOCAMPUS.

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Single unit activities were studied in the hippocampus of the lightly anesthetized and immobilized rabbits. Of 163 units recorded during the spontaneous and induced theta activity, 94 units showed changes simultaneously with the appearance of the theta activity. They were classified into two types. The first type (74 units) exhibited a rhythmic 3 to 8 - spike burst activity recurring at 4.4/sec when the theta appeared. But it became irregular when the theta disappeared. In contrast, the second type (20 units) showed tonic single spike discharges during the theta activity, but exhibited rhythmic burst discharges (2.1/sec) in the absence of the theta. The 1st. type was suppressed by entorhinal, septal, fornical, and contralateral hippocampal stimulation for 196 - 468 msec. The 2nd. type was suppressed by septal and entorhinal stimulation for about 250 msec, but showed unstable or no responses to other stimulation. According to the depth profile of the amplitude of theta wave and marking method, the 1st. type was located in CA1, CA4 and the dentate, while the 2nd. type was restricted in CA4 and the lower blade of the dentate.

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DISTRIBUTION OF POTENTIALS PRECEDING SELF-PACED AND VISUALLY INITIATED HAND MOVEMENTS IN DIFFERENT CORTICAL AREAS OF THE MONKEY. GEMBA, H. and SASAKI, K. Institute for Brain Research, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606

A monkey was trained to lift a lever by its hand at self-pace or in response to a light stimulus, and field potentials prior respectively to self-paced and visually initiated movements were recorded in various areas on the dorsolateral and the mesial surface of the cerebral hemisphere, with electrodes implanted chronically on the surface and in 2.5-3.0 mm depth of respective cortical areas. Slowly rising, surface-negative, depth-positive (s-N, d-P) potentials were obtained prior to the self-paced movement in the bilateral premotor, and contralateral forelimb motor, forelimb somatosensory and supplementary motor cortices, resembling "readiness potentials". Potentials preceding the visually initiated movement occurred in more cortical areas than the self-paced movement. In a part of the prefrontal cortex and in the prestriate cortex on both sides, early s-P, d-N and following s-N, d-P potentials were obtained. Only early s-P, d-N potentials were recorded bilaterally in the premotor cortex. In the contralateral forelimb motor area, early s-P, d-N and late s-N, d-P premovement potentials were observed, the latter being induced by impulses via the neocerebellum and the thalamus. In the supplementary motor area, s-P, d-N and following s-N, d-P potentials were recorded at a little longer latency and with smaller amplitude than the motor cortex.

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COMPARISON OF NEURONAL ACTIVITIES IN PRECENTRAL AND SUPPLEMENTARY MOTOR CORTEX TO SENSORY SIGNALS TRIGGERING OR NOT TRIGGERING A KEY PRESS MOVEMENT OF THE MONKEY. TANJI, J. and KURATA, K. Dept. of physiology, School of Medicine, Hokkaido University, Sapporo 060

Three Japanese monkeys were trained to respond differently to two sets of sensory signals. In half of trials they were required to press a key in response to a vibratory stimulus of 40 Hz given to the hand and not to respond to a tone burst of 1000 Hz and in the other half they had to press the key in response to the tone burst, ignoring the vibratory stimulus. EMG recordings confirmed the absence of any muscle activity when required not to press the key. 3 major findings emerged: (1) Precentral neurons (PCNs) responded to the auditory signal only when the monkey pressed the key and the response had a close time relation to the motor onset. (2) only a subgroup of supplementary motor area neurons (SMANs) responded similarly as the PCNs. Another SMANs responded to the auditory signal regardress of whether the signal triggered the movement. Still other SMANs fired only when the animal ignored the signal. (3) PCNs exhibited time-locked response to the vibratory signal. The magnitude of the tactile response was greater when the signal triggered the key press. Some SMANs responded preferentially to the vibratory signal only when the animal was required not to press.

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LONG TERM UNIT RECORDING IN THE MONKEY ORBITOFRONTAL CORTEX DURING EMOTIONAL AND FEEDING BEHAVIORS. YAMAMOTO, T., OOMURA, Y., NISHINO, H., AOU, S., NAKANO, Y. and NEMOTO, S. Lab. of Humoral Control, Nat. Inst. Physiol. Sci., Okazaki 444; Dept. of Physiol., Fac. of Med., Kyusyu Univ., Fukuoka 812

Monkey with orbitofrontal cortex (OBF) lesion show changes in emotional and feeding behaviors. To elucidate neuronal mechanism involved in emotional and feeding behaviors, long term recording of single unit activity (4-48h) was analyzed in the monkey OBF during the presentation of palatable (raisin, sweet potato, bread etc.), aversive (toy snake, injector, stick etc.) and nonsense stimuli (laboratory instruments etc.) and during high fixed-ratio bar-pressing task, using teflon-coated Platinum-Iridium microwire electrode. Of 45 neurons recorded in two monkeys, 26 neurons (58%) showed phasic responses to palatable or aversive stimuli, seven neurons (16%) showed tonic responses in which those spontaneous discharge rate increased during palatable stimuli trials and decreased during aversive ones. Responses tended to diminish during repeated trials and to be modified by the monkey's attitudes toward the stimuli. These results suggest that OBF neurons are involved in the evaluation of the emotional situation as well as that of emotional stimuli.

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CYCLIC AMP RESPONSE TO NOREPINEPHRINE IN SLICES FROM RAT CEREBRAL CORTEX WITH IRON-INDUCED EPILEPTIC FOCUS. MORIWAKI,A., HATTORI,Y., INABA,K*., NISHIDA,N., YASUHARA,H. and HORI,Y. Dept. of Physiol., Okayama Univ. Med. School, Okayama 700 and Dept. of Cell Biol., Hyogo Univ. of Teacher Educat., Hyogo 673-14*.

Coritcal epileptic activities induced by a microinjection of ferric chloride solution into the left sensorimotor cortex of rats were classified into three patterns: isolated spikes appeared dominantly on the side of primary focus, those on the side of secondary focus, and bilateral spike and slow wave complexes. Relating these patterns of epileptic activities, cyclic AMP response of slices to norepinephrine was compared among several cortical regions. In animals showing the spike and slow wave complexes 30 to 60 days after the injection, the response was significantly higher on the left frontal region including the injection site than on the contralateral one. In frontal cortical regions of animals showing the isolated spikes, the response was higher on dominant side in the spike appearance than on the other side several days after the injection. However, the laterality of the response was reversed at later stage.

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PUPILLOCONSTRICTION AND THE CLARE-BISHOP AREA OF THE CAT. BANDO,T., Dept. Physiol., Yamanashi Med. Sch., Tamaho, Nakakoma, Yamanashi 409-38.

By microstimulation of the restricted region of the Clare-Bishop area, a visual area of cat occipital cortex, pupillary constriction was evoked. The area of pupil was measured by the change of infra-red light reflected from the left iris, which was averaged 20 times by using a laboratory computer. A tungsten-in-glass microelectrode with exposed tip of 50-100 um was systematically inserted along the right middle suprasylvian sulcus (MSS) with the step of 1-2 mm. In each electrode track, stimulation was done every 0.5-1 mm step until the electrode reached the bottom of the sulcus. A train of 30 bipolar pulses(amplitude, 100 uA and duration, 0.2 msec) was used for stimulation. The electrode tracks were confirmed in the histological sections by markings made during experiment.

Pupilloconstriction was evoked in the upper part of the medial bank of the central MSS, extending rostrocaudally from stereotaxic coordinate A4.5 to A2.5. Rostral to this area, pupillary dilatation was found.

This newly found pupilloconstriction area was a part of the area, in which lens accommodation was evoked by microstimulation, and lens accommodation-related neurons were found by unit recordings. The relationship of this area with the near response was discussed.

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TASK-RELATED RESPONSES OF MONKEY VISUAL CORTICAL NEURONS DURING FIXATION TASK . WATANABE, J., INAMI, T. and IWAI, E. Dept. of Behavioral Physiol., Tokyo Metropol. Inst. Neurosciences, Fuchu-shi, Tokyo 183 .

To examine the behavioral modulation on neuronal activity of striate (ST), prestriate (PREST) and inferior temporal (IT) cortices, we recorded and analysed single units of these areas of rhesus monkey performing fixation task. Many neurons, 41 % of fully tested, showed various task related responses and were classified into three types ; Sustained type (31.7 %) showing sustained inhibition or excitaion during fixation period, Transient type (8.3 %) responding with brief burst at trial start/end , and Mixed type (60.0 %). To prove these responses to be independent on light on/dim/off of fixation spot itself or on the eye movement of the animal and accompanying retinal image flow, we tested two task variations. The neurons, which we defined as task-related here, showed no response at fixation spot-off/-on in the midst of fixation or at sudden change of fixation spot location (saccade task). The ratio of task related units was 29 % in ST, 40 % in PREST and 50 % in IT, where most of the responses were mixed type. We may conclude that the modulation by task or behavior already exists in the primary visual cortex, and from where to the association cortex it gradually increase and changes its property.

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IDENTIFICATION OF NEURONS AND GLIAL CELLS AND THEIR MEMBRANE PROPERTIES STUDIED IN IN VITRO PREPARATION OF CAT'S VISUAL CORTEX. KATO, H., *KOJIMA, H. and *OGAWA, T. Dept. Physiol., Yamagata Univ. Sch. of Med., Yamagata 990-23. *Dept. Physiol., Akita Univ. Sch. of Med., Akita 010.

To assess the physiological criteria for identifying neurons or glial cells, membrane properties of penetrated cells in thin sliced tissues prepared from cat's visual cortex were studied and then intracellular stainings were carried out with procion yellow or HRP. All 13 cells presumed as glial cells according to the criteria(the absence of post-synaptic potentials or action potentials and the presence of slow de-polarization during repetitive stimulation) were identified morphologi-cally as glial cells. All 15 cells classified as neurons during exper-iments had morphologically characteristic configurations of neurons. Membrane properties of neurons and glial cells thus identified were as followings: resting membrane potential, 58±10 mV(Mean ± S.D., n=15) and 76±10 mV(n=32) for neurons and glial cells respectively; input resistance 42.1±34.0 M (n=14) and 3.0±1.6 M (n=21) respectively.

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VISUAL AND CEREBELLAR INPUTS TO THE LATERAL BANK OF THE MIDDLE SUPRASYLVIAN GYRUS OF THE CAT. MIYATA, H., KATO, N. and KAWAGUCHI, S. Dept. of Physiol., Inst. for Brain Res., Fac. of Med., Kyoto Univ., Kyoto 606

Visual and cerebellar inputs to the lateral bank of the middle suprasylvian gyrus(LS) in the cat were examined by intracellular and extracellular recording of units on stimu-lation of the optic chiasm(OX), the cortical area 18(VC), the LP-pulvinar complex of the thalamus(TH), the superior colliculus(SC), the pontine nuclei(PN), and the cerebellar interpositus and lateral nuclei(CN). Two hundreds and one neurons were identified as a projection neuron by antidromic activation to stimulation of PN(n=110), TH(n=52), VC(n= 11), SC(n=9), PN & TH(n=13), PN & SC(n=5), and SC & TH(n=1). Antidromic activation to stimulation of two different structures in the latter 3 groups of neurons is due to ax-onal bifurcation. Only 10% of the projection neurons recorded extracellularly were or-thodromically activated mostly by visual inputs in a broad sense(OX,VC,TH and SC) and partly by cerebellar inputs. Of 17 among 21 projection neurons recorded intracellularly, 10(59%) were responsive to the visual inputs and 3(18%) to cerebellar inputs, and the remaining 4 neurons(24%) were responsive to both inputs. Of 150 unidentified (presuma-bly non-projection) neurons recorded intracellularly, 106(61%) were responsive to the visual inputs, 32(21%) to cerebellar inputs, and 12(8%) to both inputs. Present results indicate that LS integrates visual and cerebellar informations.

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ORGANIZATION OF AFFERENT INPUT TO THE PREFRONTAL VISUAL AREA IN THE MONKEY. AZUMA, M., KOMATSU, H. and SUZUKI, H. Dept. of Physiology, Hirosaki Univ. Faculty of Medicine, Hirosaki 036

In the monkey frontal eye-field and neighboring dorsolateral part of the prefron-tal cortex, the lateral part represents the foveal region of the visual field, while the medial part the peripheral visual field. The purpose of this report is to determine projection sources to the two separate cortical areas. After identified cortical area representing either the fovea or periphery by examining the visual receptive fields of neurons in the areas, we injected a small amount of HRP (0.1 µl) into each area. When injected HRP into the cortical area representing the fovea, we found marked cells in the posterior wall of the superior temporal sulcus, the posterior part of the infero-temporal cortex, and the lateral part of the prelunate area. Marked cells for the area representing the periphery were found in the anterior wall of the superior temporal sulcus, the posterior part of the superior temporal cortex, and the medial part of the prelunate area and posterior parietal cortex. The marked cells were also found abundan-tly in the lateral rim of the dorsomedial thalamic nucleus: injection to the cortical area for the fovea gave rise to marked cells in its ventral part, while that for the periphery in the dorsal part.

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LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION IN VISUAL CORTICAL NEURONS OF IN VITRO SLICES FROM YOUNG KITTENS. KOJIMA, H. and OGAWA, T. Dept. of Physiol., Akita Univ. Sch. of Med., 1-1-1 Hondo, Akita 010.

Long-term potentiation (LTP) of synaptic transmission in the visual cortex was studied in in vitro slices from young kittens with intracellular recording techniques. Bipolar stimulating electrode was placed in the white matter and intracellular recordings were done at layer II or III with 4M K acetate-filled micropipettes. A total of 39 cells successfully impaled provided data base for this study. Following a brief tetanus (10 Hz for 30 sec) which was strong enough to evoke action spikes in impaled cells, the amplitude of EPSPs was observed to increase for as long as 15 to 70 min. or more. In some cells the reciprocal of threshold for evoking a postsynaptic action spike was enhanced for a comparable period of time after tetanus. These enhancements (long-term potentiation) were not correlated with changes in input resistance of the neuronal membrane. LTP was also observed in slices superfused with bicuculline-containing (4μM) bathing solution. The conclusion is that excitatory transmission is subjected to LTP after a brief tetanus of afferent fibers.

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DEVELOPMENT OF SYNAPTIC TRANSMISSION IN THE STRIATE CORTEX OF THE INFANT KITTEN INVESTIGATED BY A SLICE PREPARATION. KOMATSU, Y. Dept. of Physiol., Sch. of Med., Nagoya Univ., Tsurumai, Nagoya 466

Excitatory and inhibitory synaptic transmissions were studied by intra- and extracellular recordings from cortical cells using slice preparations of striate cortex of infant kittens (1-127 days old). Conduction velocities of afferent impulses were less than one-tenth of the adult level at birth, and gradually increased during several months. Stimulation of white matter produced EPSPs in all cells sampled from very young (1-9 days old) animals. In these animals EPSPs were all monosynaptic except for few cells in infragranular layer. Cells exhibiting polysynaptic EPSPs were increased gradually during the three months after birth. The increase was most remarkable in supragranular layer. Correspondingly, there was an increment in the rising slope of EPSPs during that period. No IPSP was founded in cells of very young (1-5 days old) kittens except for those in infragranular layer. The number of cells exhibiting IPSPs increased rapidly during two months after birth. There were corresponding increments in amplitude and duration of IPSPs. Extracellular study of inhibition in cortical units indicated that the development of inhibition occurs differentially according to the layer. It was earliest in layer V next in layers IV, II-III and latest in layer VI.

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RESPONSE PRORERTIES OF NEURONS TO PATTERN STIMULI IN ANTERIOR AND POSTERIOR INFEROTEMPORAL CORTEX. HIKOSAKA, K., SATO, T. and IWAI, E. Dept. Behavioral Physiology, Tokyo Metropolitan Inst. for Nerosci., Fuchu-city, Tokyo 183

Response properties to two dimentional patterns were investigated in the anterior (AIT: Area TE) and posterior inferotemporal cortex (PIT: Area TEO). Monkeys were trained to respond two of four successively presented patterns (go-no go tasks). Patterns of variant size and form and components of four patterns were also presented as rewarded or unrewarded stimuli. Sixty seven neurons in four hemispheres of two Japanese monkeys were identified to be responsive to pattern stimuli, 26 neurons were recorded in AIT and 41 neurons were in PIT. Responsiveness to four patterns or response latency to pattern stimuli was variable among the neurons in both areas. The activities of neurons in PIT were more easily affected by the change of pattern size or variants of patterns and warning tone. On the other hand, in AIT, neurons which responded selectively to one of the four patterns were usually not affected by the variants of the patterns and warning tone. These evidences suggested that AIT was different from PIT in the process of categorization or recognition of two dimentional patterns.

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STRETCH REFLEX COMPONENT OF FORCE IN RESPONSE TO STRETCHING MIDDLE FINGER EXTENSOR DIGITORIUM COMMUNIS MUSCLE IN MAN. AKAZAWA, K. and FUJII, K.* Dept. of Elec. Eng., Faculty of Eng., Osaka University, Yamada-Oka, Suita, Osaka 565

Measured force in response to stretching the muscle consists of two components; one is originated from the intrinsic muscle stiffness and the other is from the stretch reflex. The present work is to estimate the reflex component of force by processing time series of surface electromyogram(EMG). First step was to derive a nonlinear mathematical model which could explain dynamical relations between the muscle force and EMG. This was accomplished by applying GMDH(group method of data handling) to data of force and EMG which were observed when the subject voluntarily executed quick monophasic isometric contractions.The force at certain time could be estimated by using EMG data over the interval of 300 msec before that time. The model responses closely agreed with the time courses of isometric contraction(normalized standard error was 20%). Secondly, the middle finger was forcibly flexed quickly by four degrees when the tonic force was maintained isometricly. Measured stretch-evoked EMG was input to the model, and then the force of reflex origin was computed by the model on a computer. Ten responses were computer averaged. Peak of the estimated reflex component of force was about 50-100 % of the measured stretch-evoked force.

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EFFECTS OF REFLEX LOOP GAIN ON PHYSIOLOGICAL TREMOR FREQUENCIES. WATANABE, A. and SAITO, M. Inst. of Medical Electronics, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113

A new model of physiological tremor was developed incorporating a second-order mechanical component and the spinal and supraspinal reflexes. The following two parameters were introduced in the model: 1) the gain ratio of the two reflex loops (long loop gain / short loop gain), and 2) a characteristic frequency inversely proportional to the difference of the two delay times, which corresponds to a fixed point in the graph of the reflex term of the phase angle equation. Based on the model, the physiological parameters were estimated using sustained oscillation frequencies measured during slow relaxing movement of the hand with inertial loads. A doublet line spectrum (frequency splitting) observed under a specific loading condition supports the model and enabled us to calculate the parameters. In the present study the behavior of the tremor frequency is simulated as a function of the gain ratio using the parameters of the hand described above. The results of the simulation reveal that the doublet line spectrum and the considerable decrease in the tremor frequency due to muscular fatigue (Stiles, 1976, 1980) can be ascribed primarily to changes in the gain ratio. In particular, as the gain ratio increases, the doublet line spectrum appears first, and then the frequency decreases asymptotically to a certain limit.

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CUTANEOUS EFFECTS ON ANKLE EXTENSOR AND FLEXOR MOTONEURONES. Kasai,T.,Manabe,M. and Tanaka,R. Department of Neurobiology, Tokyo Metropolitan Institute for Neurosiences, 2-6 Mushashidai, Fuchu City, Tokyo, Japan

Effects of the sural nerve stimulation on the soleus (Sol) and anterior tibial (TA) motoneurones were studied by the H-reflexes and epidurally recorded spinal root potentials on 8 normal subjects. The sural nerve was stimulated at the level of the external malleolus through bipolar surface electrodes (rectangular pulses with 1msec duration and intensity less than pain threshold). The earliest were reciprocal, facilitatory on Sol and inhibitory on TA, and the shorter interval for the effects was ca, 30msec between conditioning and test stimulus. On the other hands, the earliest effects of the medial plantar (mixed) nerve stimulation was inversely reciprocal and appered at ca, 10msec. Epidural recordings of afferent volleys showed that the difference between plantar and sural afferent volleys was only 1msec. Thus, the central latency of cutaneous effect was much longer than the muscular afferent effects.

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SINGLE MOTOR UNIT ACTIVITIES DURING PROLONGED ACTIVITY.
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The peripheral fatigue, that is the failure of their peripheral excitation, and the central fatigue, that is the decrement of firing frequency, during prolonged activity at various tensions in intact human motor unit were investigated by use of the surface method. The results were as follows: 1) The interspike interval during prolonged activity at all tensions increased for first 6 min and then decreased gradually. 2) The conduction velocity during activity changed at various manners depending upon the tension. At the tension of 40 N and under, the velocity decreased. On the other hand, the tendency of the decrement seems getting smaller at the tension of 50 N and over. 3) The muscular temperature did not changed at the tension of 40 N and under, but increased at 60 N.

The conduction velocity was not to be the index of peripheral fatigue at high activity which changed the muscular temperature. There is a need in submaximal prolonged activities for recruitment of more units and an increase in the motor unit firing frequency if the same tension is to be sustained.

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VARIATIONS IN THE H-REFLEX OF SOLEUS AND TIBIALIS ANTERIOR MUSCLES IN HUMAN STANDING.
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We have been reported that the lower leg muscle activities were closely correlated with the position of centre of gravity, and that H-reflex of soleus muscle (Sol) was facilitated during forward leaning and was sharply depressed during backward leaning. In this paper, we confirmed that H-reflex of tibialis anterior muscle (TA) was evoked during backward leaning. Furthermore we investigated the effects of electrical stimulation of the common peroneal nerve (CPN) or the tibial nerve (TN) on the H-reflex responses in SOL or TA in upright standing. The conditioning CPN stimulation produced an early depression (2-20msec) and a facilitation (30-50msec) and a later depression (60-120msec) of the SOL H-reflex. The earlier depression might be due to the inhibitory effects of the antagonist group I muscle afferents. The following facilitation and depression was considered to be due to the stretch of the SOL by the antagonist muscle twitch. This triphasic pattern was also observed in the rectified EMG of SOL. By the conditioning TN stimulation, the SOL H-reflex was initialy depressed and facilitated at about 150 msec and then oscillated with dumping in a physiological tremor frequency.

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MULTIPLE REGRESSION ANALYSIS OF FACTORS CONTROLLING SOLEUS MUSCLE ACTIVITY DURING FORWARD INCLINATION OF HUMAN SUBJECTS.
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We have already reported that EMG activities of the soleus muscle is transiently suppressed during forward body inclination. We attempted to demonstrate neural events in the soleus motoneurons during the body inclination by statistical (multiple regression) analysis of the relationships of refiring time of the soleus EMG with parameters of the inclination. Among more than 10 parameters of the inclination studied, five parameters were found to significantly contribute to the refiring time. The angle of inclination 50 ms before the refiring was the strongest factor to determine the refiring time (regression coefficient = -0.26). The 2nd was the angle at the time of the refiring (0.22), the 3rd mean velocity of inclination (-0.19), the 4th over-all angle of inclination (0.17), and the last velocity of inclination at the time of the refiring (0.01). These results indicate that the parameters controlling the soleus activity are 1) the proprioceptive inputs fed back from the ankle joint and the muscles related to the inclination (the 1st, 2nd and 5th factors) and 2) central inhibition (3rd and 4th factors).

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ADRENERGIC MODULATION OF THE THELAMOCORTICAL SOMATOSENSORY PATHWAY FROM THE LOCUS COERULEUS. MISHIMA, K. Dept. of Physiology, Fac. of Dentistry, Kyushu University, Fukuoka 812

Effect of conditioning stimulation of the locus coeruleus (LC) was examined on the somatosensory cortical (Ctx) and the ventrobasal thalamic (VB) evoked potentials which were induced by test stimulation of the white matter and the medial lemniscus, respectively. Stimulation of the LC increased the Ctx evoked potential and reduced the VB potential.

An α-adrenergic blocker, phentolamine (2 - 4 μg) and a β-blocker, sotalol (2.5 - 10 μg) were injected into the cerebral lateral ventricle. It seemed that phentolamine antagonized LC facilitation of the Ctx potential without affecting LC inhibition of the VB potential, whereas sotalol antagonized LC inhibition of the VB potential without affecting LC facilitation of the Ctx potential.

Double pulses stimulation of the white matter augmented the Ctx potential and that of the medial lemniscus inhibited the VB potential for a prolonged period. Somatosensory transmission process in the Ctx was different from that in the VB. LC influences appeared to potentiate the facilitation in the Ctx and the inhibition in the VB and an α-adrenergic mechanism may be involved in the former and a β-mechanism in the latter.

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PROPERTIES OF SLOW COMPONENT OF PHOTICALLY EVOKED LID POTENTIAL CHANGES IN RABBIT. HOSHINA,Y., OZAKI,T. and NIKARA,T. Dept. of Physiol., Hirosaki Univ. Sch.of Med., Hirosaki and Lab.of Physiol., Sch.of allied med., Hirosaki Univ., Hirosaki

In order to clarify the origin of slow component of potential changes in the eyelid elicited by flash stimulation in rabbit, the average summation responses of photically evoked lid potential changes, EEG, ERG, EOG and MV responses were recorded simultaneously under various conditions and studied polygraphically.

In majority of cases with unilateral anophthalmos, the slow component of photically evoked lid potential changes appeared in extirpated side and was decreased markedly in the contralateral side. This fact seems to be due to anatomophysiological characteristics in rabbit. In some cases, it disappeared in extirpated side and appeared in contralateral side. The slow component was recognized to correspond to the late response of the VEP, which is reported to disappear in moderately nembutalized rabbit. In addition, the slow component was decreased slightly by the administration of ketamine in smaller doses, although the early component of photically lid potential changes was decreased markedly or disappeared. The early and slow components were decreased considerably by administration of ketamine in moderate doses.

The above results suggest that the slow component of photically evoked lid potential changes is related closely to the late response of the VEP.

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ELECTROMYOGRAPHIC COMPONENTS OF PHOTICALLY EVOKED LID POTENTIAL CHANGES : ESPECIALLY REFERENCE TO ORBICULARIS OCULI REFLEX. TAKEO,T., OZAKI,T., HOSHINA,Y., SUGA,S. and TERAMOTO,S. Dept. of Physiol., Hirosaki Univ. Sch. of Med., Hirosaki and Nagasaki National Central Hospital, Nagasaki

In healthy subjects and patients with anophthalmos or unilateral facial palsy, the electromyographic (EMG) components of average photically evoked lid potential changes obtained bipolarly under the various conditions were studied polygraphically from the standpoint of orbicularis oculi reflex.

According to the level of cortical activity, the EMG components of average photically evoked lid potential changes were decreased gradually and they disappeared in stage 2 of natural sleep. In the course of dark adaptation, they were increased in amplitude and shotened in latency. With the intensity of the flash stimulus, their amplitude and latency were increased and shortened respectively. In the occulded lid of healthy subjects and in the impaired lid of patients with an artificial eyeball, they were observed to be decreased slightly. In the impaired lid of patients with unilateral facial palsy, they were decreased or disappeared corresponding to the severity of paralysis.

From the above results, it was concluded that the EMG components of photically evoked lid potential changes reflected the activities of the effector in orbicularis oculi reflex and were the most useful indicator to evaluate them.

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AN ANALYSIS OF THE NONLINEARITY OF THE VISUAL EVOKED RESPONSES OF THE CATS.
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In the present paper, the nonlinear characteristics of an oscillation generating system of the visual evoked responses(VER) were investigated from the mathematical viewpoints. The flashlight stimulation frequency (S.F.) were changed stepwise between 1 and 65Hz in increasing and decreasing order. Amplitude change and phase change of each harmonics of VER were examined. Two amplitude entrainments were observed :one consisted mainly of the 2nd.harmonics near 20Hz of S.F.,the other consisted of the 2nd subharmonics near 40Hz of S.F. In most case ,the same reluctant type hysteresis of the amplitude of the 2nd harmonics were observed both in cortex and lateral geniculate body near 20 Hz of S.F.. Near this entraiment frequency of S.F.the phase of the 2nd.harmonics revealed sudden jumps,while it made monotonous change before and after the jumps. The amplitude of them made great changes at the S.F.of phase jumps. The orbits of VER on the phase plane exhibited the different types of limit cyles before and after the phase jumps. From these facts, it may be concluded that there exist at least two kinds of limit cycles in this oscillation generating system.

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TOPOGRAPHY OF OFF RESPONSE OF VISUAL EVOKED POTENTIAL
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Using flash as stimulation, thus obtained visual evoked potential included on and off responses and those two could hardly be separated. By employment of long lasting light such as radiance of LED, off response were recorded which had peak latencies of 200 msec (positive) and 250 msec (negative) after the turn off the light. In the previous study the off response tended to be larger at parietal than at occipital recording point. In order to verify this tendency isopotential figure(IPF) was drawn by Ashida's polynomial interpolation method. Subjects were healthy medical students. Two lines (10 cm long and 5 cm separeted) of 40 LEDs placed 60 cm apart from the subject face emitted red light of 1125 msec duration. The stimulation was applied every three seconds and repeated 100 times. IPFs at the times of the peak latencies were compared with "composed" IPF and "averaged" IPF. The IPFs seemed to support the above-mentioned tendency.

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KALMAN FILTERING OF VISUALLY EVOKED CORTICAL POTENTIALS WITH PROCESSING PROCEDURES FOR COLORED NOISE.
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The Kalman filtering technique in which the background EEG was assumed as a gaussian white noise was applied to detect the visually evoked cortical potentials (Morita et al., J. Physiol. Soc. Jpn. 40: 263, 1978, Nakamura et al., Jpn. J. Med. Electron. Biol. Eng. 17: 409, 1979). The filter was improved considering the colored noise of the EEG. EEG was approximated as the output of a linear dynamic system being drived by white noise, because the stochastic property and frequency spectrum in response to the pattern reversal stimuli were independent of the visual acuity. Three types of shaping filters were formulated, (1) 1st order linear system, (2) 2nd order linear system, and (3) four 2nd order linear systems. It was concluded that only 10-20 averaging times were necessary to detect the VECPs, and the most suitable shaping filter was (2).

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INCREASED INTRACRANIAL PRESSURE (ICP) AND AUDITORY EVOKED POTENTIALS
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Increased ICP produces suppression of various neural activities. Each component of auditory evoked responses is considered to represent the activities of topographically different neuron group. We investigated changes in auditory evoked responses to an elevated ICP to analyse relation between ICP and the degree of neural dysfunctions. When ICP was elevated by inflating a small balloon buried in the intracranial space, auditory evoked cortical response (AECR) decreased in amplitude. The depression occurred first in the late components. Decreases in response size at medial geniculate body and inferior colliculus followed after depression of AECR. Responses of cochlear nucleus were not depressed by an elevation of ICP even when AECR almost disappeared. However, a decrease in the responses of the lower brain stem was observed when marked pressor response was induced in addition to highly elevated and sustained ICP. The observations suggest that AECR is most vulnerable to increased ICP and among different relay stations of the ascending auditory system, higher relay nuclei were affected more severely than the lower ones.

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Influence of methylguanidine (MG) on the electrical phenomena in the central nervous system. Sawada, M., Naito, H. and Yasuhara, M. Dept. of Physiol., Kansai Med. Univ. Moriguchi, Osaka 570

In the attempt to clarify the pathophysiology of uremia, electrophysiological experiments were carried out. MG was injected intravenously in the rabbit and the followings were observed. I. There was a rise in the thresholds of the arousal reaction and evoked muscular discharges (EMG) due to stimulation of the brain stem reticular formation (RF) and a reduction in the frequency of spontaneous unit discharges of RF. 2. A rise in the threshold of EMG on stimulation of the cerebral cortex but a decline in that on stimulation of the hippocampus. 3. The amplitude of the late component of the afferent average evoked potentials due to stimulation of the sciatic nerve decreased while the amplitude of N_3 increased. 4. The amplitudes of the nociceptive reflex and M-H wave decreased. 5. Spontaneous discharge of the sympathetic nerve was intensified. 6. The blood flow volume of common carotid artery showed a trend to decrease. 7. Intestinal movement was inhibited. 8. Microvibration was facilitated. 9. Photo palpebral reflex (PPR 6, 7, 8) incresed in amplitude.

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STUDY OF LATE COMPONENT OF PPR IN RABBITS. *YASUHARA, A., YAMADA, A., NAITO, H. and YASUHARA, M. Dept of *Pediatrics and Physiology, Kansai Medical University, Moriguchi, Osaka 570

The orbicularis oculi muscule contracts in response to photic stimulation as a reflexive action, and it is called the photopalpebral reflex (PPR). The purpose of this study is to clarify the origin of the late component of PPR which is called PPR_9. The animals used were 49 Japanese white rabbits weighing 2.0-3.0 kg. For recording PPR, silver electrodes were placed on the center of the upper eyelid and the both ears for single polar recording. The average of 50 responses was calculated. The latency of PPR_9 is about 200 msec. The late response of visual evoked response (VER_5) was observed to have the same latency as PPR_9. After decortication using 25% KCl solution VER_5 disappeared while PPR_9 was reduced without disappearance. Elimination of cerebral cortex suppressed PPR_9 and after removing hippocampus with cerebral cortex PPR_9 disappeared. It is concluded that the late component of PPR (PPR_9) is the potential which originates in both cerebral cortex and hippocampus.

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THE RESPONSIVENESS OF THE FREQUENCY COMPONENTS OF CHILDREN'S EEGS TO THE LIGHT STIMULUS.
KATADA, A., HORIE, M., SUZUKI, H.,* ICHIMURA, K.* and KOIKE, T.** Lab. of Physiopsychol., Tokyo Gakugei Univ. Koganei, Tokyo, * Lab. of Physiol. for the Developmentally Handicapped, Ibaraki Univ., Mito, Ibaraki, ** Dept. of Education, Tohoku Univ., Sendai.

We have pointed out that the statistical property of the variability of theta and alpha frequency components was different each other on the developmental changes of EEG. Because of the marked response to the light, alpha component is considered to have the close relation to the visual function. We studied the responsiveness of theta component to the light stimulus. EEGs were led monopolarily along the midsagittal line. The stimulus was a white dim light of 50 Lux, the duration of which was 30 sec and presented 50cm apart from the eyes, 5-9 times at the interval of about 60 sec . The analysis of EEG was done through the band pass frequency analyzer and the minicomputer(PDP 11/34). In the band pass analysis, the theta filtered wave responded later and recovered faster comparing with the alpha one, and in some subjects the theta filtered wave did not respond markedly. Through the digital computer, auto-power spectra of EEGs were calculated. Both theta and alpha frequency components did not syncronized on the time course, and showed different recovery processes each other. From these results, the origin of theta component might be different from that of alpha one.

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SHORT-LATENCY SOMATOSENSORY EVOKED POTENTIALS INDUCED BY SUBCUTANEOUS DEEP TISSUE STIMULATION.
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Subcutaneous deep tissue near the right ulnar periosteum in humans was stimulated by an electric pulse of 0.2 msec in duration. The averaged somatosensory evoked potentials (SEPs) were led from the scalp portion (C3') corresponding to the left somatosensory cortex. A pair of stimulating electrodes were of Teflon-coated stainless acupuncture needles with a diameter of 0.2 mm. Each tip was left uncoated. They were inserted onto the periosteum with an interelectrode distance of 2 to 3 cm. To obtain short-latency SEPs, the reference electrode was placed on the left hand and averaging triggered by a pulse of 400 to 500 msec after the R wave of EKG was needed. The response from C3' is considered to be both cephalic and non-cephalic in origin, but that from cerv 4 (cervical 4 spine) may be non-cephalic. P40 and N50 are cephalic, while P25 may be non-cephalic. They disappeared after an injection of 2% Xylocaine. Thus, the short-latency SEP obtained seemed to be only P25 component.

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ENHANCEMENT OF INHIBITORY EEG SPIKES IN THE KINDLED HIPPOCAMPUS OF THE RABBIT.
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In the kindled hippocampus there exist EEG spikes, whose intracellular correlates are hyperpolarizations of pyramidal cells (referred to as inhibitory EEG spikes, Fujita et al., Jpn. J. Physiol., 31 (1981) 879-889, ibid., 33 (1983) in press). In fact, interictal spikes are inhibitory EEG spikes. When the brain stem including the median raphe nucleus was stimulated with pulses at 50/sec, the EEG theta rhythm was replaced with fast waves followed by inhibitory EEG spikes. The intracellular correlates of these EEG spikes were Cl-non-dependent hyperpolarizations of pyramidal cells. These inhibitory EEG spikes were conspicuous in the kindled hippocampus. Stimulation of the contralateral hippocampus with pulses at 0.8-1.3/sec elicited inhibitory EEG spikes at a latency of 600-700 msec. The corresponding hyperpolarizations of pyramidal cells consisted of both Cl-dependent and Cl-non-dependent components. It is only in the kindled hippocampus that stimulation of the contralateral hippocampus produced inhibitory EEG spikes. These inhibitory EEG spikes would doubtless counteract excessive excitation, i.e. seizure, constituting a part of the protective mechanism for the brain.

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LAMINAR ANALYSIS OF HIPPOCAMPAL THETA WAVES OF THE CAT.

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We have recently observed that electrical stimulation of the lower part of the septum induces rhythmical slow waves (theta rhythm, 3-4 Hz) in cats. In this study, we attempted to analyze the generator zones of theta waves in the dorsal hippocampus in cats lightly anesthetized with urethane (initial dosage of 100-200 mg/kg, I.V.). Laminar analysis of the theta waves was made using a bundle electrode, which was made of four tungsten wires of 150 μm diameter insulated except the tips. Monopolar recordings of the theta waves were made simultaneously from four different depths, with the reference electrode placed on the stereotaxic frame. Locations of the electrode tips were verified histologically at the end of the experiments. Depth potential profile showed the two peaks in amplitude of the theta waves. One smaller peak was located around the pyramidal layer (maximum amplitude 100-200 μV). The larger peak was located in the dentate gyrus (maximum amplitude 200-350 μV). It was demonstrated that the two theta waves, the one around the pyramidal layer and the other in dentate gyrus, were approximately 180° out of phase. These results may support the 'two generator hypothesis' of theta waves.

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CROSS-CORRELATION OF HIPPOCAMPAL UNIT ACTIVITIES DURING SLEEP-WAKING CYCLE. Eguchi, K., Bengelloun, W. A.* and Satoh, T. Dept. of Physiol., Sch. of Dental Med., Aichi-Gakuin Univ., Nagoya 464 and Dept. of Biol., Fac. of Sci., Univ. of Mohammed V, Morocco*

Three tungsten microelectrodes were driven simultaneously into the hippocampus of the chronically prepared, undruged cats sitting under painless head restrainer. When the electrodes were in the stratum radiatum of the regio superior, several positive waves of 0.2-0.3 mV in amplitude and 10 ms in duration were recorded to occur in burst occurred in burst and in a good synchronization at different sites. Their occurrence was irregular during slow wave sleep (46/min), very rare during wakefulness (7/min), and totally absent during REM sleep. Spike discharges sometimes could occur at the junction of two consecutive positive waves. 13 pairs of neuronal activities were recorded during different sleep-waking phases and their temporal auto- and cross-correlations were measured. Synchronized discharge was the most often observed relationship between a pair of neurons throughout all phases (13 out of 21). When the background phase was shifted, the cross-correlograms showed more or less changes and never remained in the same relationship in 6 out of 8 cases, suggesting a strong, phase-dependent modulation of the mode of information processing. Changes in the auto-correlogram were less striking; one half of the neurons showed no apparent change. In the rest the tendency to short-interval discharge was variously modified or lost.

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INTRINSIC SLEEP-WAKING PATTERN IN CATS UNDER CONSTANT LIGHT AND CONSTANT DARK SCHEDULE. KUWABARA,N.*,AOKI,K.*, SEKI,K.**,MIZUSHIMA,Y.** and TAYA,Y.**
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In the last study we reported the daily variation of sleep and waking in cats under light and dark schedule(LD 12:12). Cats showed multiphasic sleep-waking pattren having much more total sleep time in the lighted period than in the dark period. In the present study we recorded polygraphically the intrinsic sleep-waking under constant light and constant dark schedule. The typical sleep-waking pattern once observed in the LD schedule still remained both in the initial of constant light and constant dark schedule respectively. However, the sleep-waking cycles tended to be relatively monotonous throughout a day. In the constant dark schedule the amount of waking time incresed throughout a day especially in the period correspond to lighted period in the LD schedule. In the constant light schedule the amount of total sleep time slightly incresed and PS relatively decresed as time goes on.

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EFFECTS OF A NEW ELECTROSLEEP DEVICE ON SLEEP. MORITA, Y., SENO, H., NAGATA, K.*, ISHIKAWA, N., MATSUMOTO, J., MORI, K.*1 and MIYAMOTO, H.*2 Dept. Physiol., Sch. Med., Univ. Tokushima, Tokushima 770, *1Inst. Defectol., Univ. Tsukuba, Ibaraki 305, *2Inst. Med. Engng., Tokyo Women's Med. Coll., Tokyo 162

We have suggested that the suppression of sympathetic activity might be a sleep-initiating factor through animal experiments using cats. Based upon the animal experiments, we have developed a new device of electrosleep, which is designed to generate square wave pulses (3-4V, 0.2msec), the frequency of which is repeatedly varied from 14 to 1 Hz every 3 minutes. The device was used in experiments with normal adults and insomniacs to evaluate the influence of electrosleep on sleep. With normal adults, the effectiveness of the device in reducing daytime sleep latency was confirmed by the cross-over procedure. In the case of insomniacs, significant improvements were obtained in the three items : sleep latency, soundness of sleep, and mood at morning awakening. Moreover, a rise in the hand skin temperature during electrosleep was visually displayed by thermography as a result of vasodilatation in the hand skin due to a decrease in sympathetic tone. From these, the device is considered effective in inducing sleep and its clinical application is useful for insomniacs characterized by difficulty in initiating sleep, whether in transient or persistent DIMS (Sleep : Vol. 2, No. 1, 1979).

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MARKOV PROPERTIES OF SINGLE NEURONAL ACTIVITIES DURING SLEEP-WAKING STATES. YAMAMOTO, M. and NAKAHAMA, H. Div. of Neurophysiology, Inst. of Brain Diseases, Tohoku University School of Medicine, Seiryo-cho, Sendai 980

We compared renewal and Markovian characteristics of neuronal discharge sequences in the somatosensory area I (SI) and the mesencephalic reticular formation (MRF) of the head-restrained cats during a sleep-waking cycle. The profile of the pooled renewal properties for the shorter interspike-interval component predicted by the exponential tail of interval distribution during a sleep-wakefulness cycle was quite different from that for the total intervals including the longer ones. In the SI neurons, group mean of the degree of Markov properties of the shorter intervals was larger during active wakefulness and REM-sleep without PGO waves. In the MRF neurons, the similar tendency as in the SI was observed. Furthermore, the degree of Markov properties of them was much higher than that of the SI neurons irrespective of the sleep-waking states. From the above results, it is suggested that the non-Markovian activities during quiet wakefulness and REM-sleep without PGO waves may be regarded as autochthonous ones; and that the MRF neuronal group has a tonically modulated unit activity even in the absence of external stimuli.

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CHANGES IN MULTIPLE UNIT ACTIVITY PRECEDING SLEEP-WAKEFULNESS IN THE RAT. HANADA, Y. and KAWAMURA, H. Lab. Physiological Psychology, Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194.

For the purpose of evaluating roles of the hypothalamus and pons in sleep-wakefulness regulation, multiple unit activities (MUA) were recorded from various brain areas in intact rats. Three sleep-wakefulness stages, wakefulness, slow-wave sleep and paradoxical sleep were specified by visual inspection of polygraph records of ECoG and EMG from neck muscles.

The time lags between changes of MUA and changes of ECoG at the onset of each of these sleep-wakefulness stages were measured in 50 MUA recording sites. Among them 11 sites were in the anterior hypothalamus (AH), 12 sites in the posterior hypothalamus (PH), 7 sites in the thalamus and 20 sites in the midbrain and pons.

Among 9 sites which showed the earliest changes in MUA, 4 sites were located in AH, 4 sites were in PH and only one site was in the midbrain. Early changes was observed more frequently in AH and PH than in the midbrain and pons.

These results suggest a possibility that the hypothalamus plays more important role in regulation of sleep-wakefulness rhythm than the hindbrain.

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CORRELATION BETWEEN PARADOXICAL SLEEP AND LIFE SPAN. NAGASAKI,H Dept. of Physiology, Yamanashi Medical College, Tamaho, Nakakoma, Yamanashi 409-38

In order to know a correlation between an daily amount of paradoxical sleep and a mean life span (50% survival), the amount of paradoxical sleep was measured in an long-life span inbred strain of mice habituated in three different circumstances. A group habituated in a shattle box with a electric foot shock from 3 month to 15 month showed a short life span (7 month) and a short daily paradoxical sleep(81 $\pm$ 4 min / day) as compared to a special pathogen free circumstance. The special pathogen free group showed a long life span (21 month) and a long paradoxical sleep (106 $\pm$ 4 min / day). A group without electric foot shock habituated in a shattle box showed a short life span (13 month) and a short paradoxical sleep (80 $\pm$ 4 min).

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CHANGES ON WATER REINFORCEMENT LEARNING TEST OF TRYPTOPHAN DEFICIENT FEEDING RATS. OKAMURA,K.,OHTSUJI,M.and NOMURA,M. Dept. of Physiol. School of Med. Fujita-Gakuen Univ. Toyoake, Aichi 470-11, JAPAN.

Newborn rats were fed on a tryptophan deficient diet. The retardation of body and brain growth was remarkable. The physical growth recovered under a tryptophan therapy supplemented to the tryptophan deficient diet. Three months old rats of the tryptophan deficient and the tryptophan supplemented therapy were subjected to a brightness discrimination learning test of an operant procedure, using a food pellet (45mg) as a reinforcer on food deprived condition. A 1000:1 brightness was discriminated, the brighter stimuli on variable interval 15 seconds schedule were reinforced and the darker stimuli were not reinforced. The tryptophan deficient rats did not exceed 80% on correct response ratio until the 30th session, whereas the normal and the tryptophan supplemented rats scored more than 85% on the 20th session. Three groups of the rats were subjected to the same procedure, using a water dip as a reinforcer on water deprived condition. The tryptophan deficient rats scored less than 80% on correct response ratio until the 30th session, whereas the normal rats scored more than 85% on the 20th session, and the tryptophan supplemented rats scored more than 85% on the 21st session. These behavioral changes of the tryptophan deficient rats correlated with the retardation of the serotonergic nervous system.

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CHANGES IN LEARNING PERFORMANCE IN RATS WITH SPONTANEOUS HYPERTENSION. OHTSUJI,M.,OKAMURA,K. and NOMURA,M. Dept. of Physiol. School of Med. Fujita-Gakuen Univ. Toyoake, Aichi 470-11, JAPAN.

Spontaneous hypertensive rats(SHR) are very sensitive and hyperactive to strong stress such as a foot-shock, so we subjected more natural learning schedule than negative reinforcement. SHR reached a high level of performance in a Fixed-ratio 50 schedule, the inter-reinforcement interval and the post-reinforcement pause were shorter, and the response rate per minute was higher in SHR than those of WKY. The contents of norepinephrine(NE) showed very low levels in lower brain stem levels with nucleus tractus solitalii (NTS) of SHR. Dopamine showed no difference between SHR and WKY of any other brain areas.

The fluorescence histochemical analysis was performed with the glioxylic acid method according to faglupagas fixation. The fluorescent materials in the axons of NTS, which contained afferent fibers of the baroreceptor reflex arc, showed no accumulation.

The facts of hyperactivity in behavior, hypertension in blood pressure and the reduction of NE in NTS indicated that the number of catecholaminergic fibers was decreased in hypertensive stage of SHR.

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STUDIES ON NEUROTRANSMITTER CANDIDATES IN ANTERIOR FOREBRAIN ROOF CONTROLLING IMPRINTNG BEHAVIOR IN CHICK. TAKAMATSU, K. and TSUKADA, Y. Dept. of Physiology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160

A series of experiments has implicated the medial hyperstriatum ventrale (MHV) in imprinting and we confirmed these findings using ^{14}C-2-deoxyglucose method and by lesion study. The present study was conducted to examine the neurotransmitter candidates in MHV region controlling the imprinting behavior. Chicks were matched in pairs according to the basis of their following responses. Inhibitory drug of neurotransmission such as 6-hydroxydopamine, haloperidole, atropine, α-bungarotoxin and kainic acid was injected into MHV region bilaterally. After the injection, following response was observed. When 6-hydroxydopamine (50μg in 1μl) was injected into MHV, the motor activity was supressed and the following response was greatly diminished. The same effect was observed by the administration of haloperidole. On the other hand, the injection of atropine (20μg) caused the significant impairment of the following response, but the moter activity was not affected. The injection of α-bungarotoxin did not show any effects on both the following response and motor activity. By the administration of kainic acid (10^{-5}M) or glutamic acid (10^{-3}M), the following response was significantly impaired but the motor activity was not affected. From these results, it was suggested that muscarinic cholinergic and glutamatergic synapses in MHV might have some roles in imprinting behavior.

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VISUAL TRACKING OF THE STEP-LIKE TARGET SHIFT IN THE MENTALLY RETARDED CHILDREN. SUZUKI, H., OZAKI, H. and ICHIMURA, K. Lab. of Physiol. for the Developmentally Handicapped, Ibaraki Univ., Mito 310

Characteristics of the eye movement in the mentally retarded children were studied by measuring electro-oculogram (EOG) in reference to the visual tracking of the horizontal step-like shift of a target. Similarly to the normal children under this condition, the saccade was prevailing also in the retarded. The reaction time was of ordinary values, ie., the mean in the most retarded subjects was distributed between 120 and 250 ms, but individual values were dispersed over larger ranges than the normal. Velocity of the saccade showed also normal values within small angles of the shift (about 10 deg./100 ms for 5 degrees shift and about 15 deg./100 ms for 10 degrees shift), while it became relatively slower as the shift angle increased (more than 20 deg./100 ms in the normal and almost within that value in the retarded for 20 degrees shift, respectively). Staircase traces, overshoot or a standstill of EOG could be frequently observed as an unsuited pursuing, which may indicate immaturity of the target-position relocation function of the saccadic eye movement. Additionally, fixation at a static point was fluctuated. Head rotation accompanying the tracking and the associative turning back of the eye position was recorded and the occasional excessive participation of the head movement was pointed out.

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IMPAIRMENT OF PATTERN DISCRIMINATION LEARNING FOLLOWING LARGE ABLATION OF MONKEY STRIATE CORTEX. NISHIO, T. and IWAI, E. Dept. of Behavioral Physiol., Tokyo Metropol. Inst. Neurosciences, Fuchu-shi, Tokyo 183.

The effects of three types of large striate lesions on relearning of pattern discrimination, detection test for a piece of raisin and discrimination limen test for patterns of reduced sizes were compaired. The 1st and 2nd lesions were removal of the representation areas of central 30 and 60 of the retina, and the 3rd one was that from 20 to 90. While the subjects (Ss) with the 3rd lesion showed no deficit in any task, the Ss with the 2nd lesion did show significant deficit in all tasks. The Ss with the 1st lesion indicated significant impairment in raisin detection and limen tests, the degrees of which were nearly equal to those shown by the Ss with the 2nd lesion. The curve of pattern relearning by the Ss with the 2nd lesion indicated slow improvement of correct responses following long-lasting performance at chance. It may be concluded that the impairment of pattern discrimination after removal of the striate cortex of central 60 is due to a combination of disorder of visual sensory function and disconnection symptom of the transmission of visual information from striate to inferotemporal cortex which is a center of visual learning.

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BRAIN DEVELOPMENT AND AMINO ACIDEMIA IN FETAL STAGE. HIRANO, S., TAKAGI, Y. AND IWASAKI, N. Dept. of Physiology, Toho Univ. School of Medecine, Ohta-ku, Ohmori-Nishi, Tokyo

Two experimental groups of Wistar albino rats, which each dam was copmulsorily given per os L-Phenylalanine(Phe) or L-tyrosine(Tyr), were examined in order to investigate the effect of maternal metabolic abnormality of amino acid on the brain development of the litter.

Free amino acid contents of the fetal brain were changed extremely in the Phe loading group. Especially, glycine and glutamine contents showed distinct changes compared with that of the normal control. However, changes of these amino acids contents were not observed in the Tyr group. Weight of brain tissue were consistently much lower in two experimental groups than that in the control, especially marked in the Phe loading group. This group showed definitely the retardation of the learning ability. However, in the Tyr loading group, the learning ability was slightly retarded compared with that of the normal control.

The results suggest that maternal hyperphenylalaninemia causes the changes in the free amino acid content of the fetal brain and it impairs the brain development, which results in the retardation of the learning ability.

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CONDITIONED HEART RATE DECREASE IN ANAESTHETIZED RATS. SHIBUKI, K., HAMAMURA, M. and YAGI, K. Dept. of Physiol., Jichi Med. Sch., Tochigi

Conditioned heart rate responses were studied in rats under anaesthesia with α-chloralose and urethane (60 and 600 mg/kg, respectively). Conditioning stimulus (CS) of strobe flash and unconditioning stimulus (UCS) of electric shock on the tail were used to train rats. Training sessions consisted of 40 CS-UCS pairs during 10 min. Control rats were trained with UCS-CS pairs. The rats were injected intraperitoneally with anaesthetics at 0, 3 h or 1 day after the training. A testing procedure composed of 20 successive trials with CS alone was conducted between 30 and 110 min after the injection. A transient heart rate decrease accompanied by inspiratory arrest of breath occurred during or after CS, and mean amplitude of the response was maximal in the rats anaesthetized 3 h after the training. The mean amplitude in these rats was significantly greater than that in the control rats anaesthetized 3 h after the training ($P<0.02$). The conditioning procedure increased mainly the amplitude of each heart rate response with little effect on the frequency of occurrence.

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EFFECTS OF 3-ACETYLPYRIDINE ON LEARNING BEHAVIOR IN MICE
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Various regions of the brain, particularly in hippocampus, have been reported to be affected by 3-acetylpyridine (3-AP) one of the nicotinamide antagonist producing deficiency in animals. In the present work, learning behavior was tested with avoidance task by jump-box training apparatus, after a single intraperitoneal administration of 3-AP at a dose of 200 mg/Kg to adult ddN-F26 strain mice. Survival intervals were three weeks. There are no significant differences in body weight and bar holding test. Open-field activities,howevere, were significantly decreased. Histologicaly, those animals taking significantly low scores by the avoidance task resulted in complete loss of pyramidal cells almost exclusively to area CA-3 in the anterior part of hippocampus.

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CONDITIONAL REFLEX DURING VARIOUS STAGES OF SLEEP IN CATS. SANO, A., MATSUMOTO, J. and ISHIKAWA, N. Dept. Physiol., Sch. Med., Univ. Tokushima, Tokushima 770

The present report is concerned with an attempt to study whether the salivary conditional reflex which had been established during the waking state can be induced in the sleeping state. Electrical stimulation of the nucleus ventroposterolateralis of the thalamus (VPL) was used as the conditional stimulus (CS), in order to avoid "occlusion" to an external stimulus during paradoxical sleep (PS).

Previously, we reported that the conditional salivary reflex had been induced successfully in slow wave sleep (SS) but it had not been elicited during PS. In the present study, considering the reports that the dream of human beings during REM sleep may be derived from the long-term memory, cats which were continuously trained according to Pavlovian salivary conditioning procedure from infancy through adult life for about 4 years were tested in the various stages of sleep. As the results, conditional salivary reflex was induced during PS as well as SS. In addition, the spontaneous salivation. which had never been observed before the conditioning, appeared during SS and PS after the establishment of the conditional reflex.

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ENTRAINMENT OF CIRCADIAN RHYTHM IN BLINDED PUPS BY NURSING MOTHER.
TAKAHASHI,K., MURAKAMI,N., AND SASAKI,Y. Dept. of Medical Chemistry, Tokyo Metropolitan Institute for Neurosciences, Fuchu-city, Tokyo 183

To determine the important role of the nursing mother in entraining the circadian rhythm of blinded pups, free-running patterns of running activity were observed during 4-5 weeks after weaning.

Blinded pups, optically enucleated on day 1, were raised by either an intact natural mother, or an intact foster mother with a rhythm reversed to that of the natural mother. The locomotor activity was continuously recorded for 4 to 5 weeks, starting between 3 and 5 postnatal weeks. At the 5th week the offset time of activity was found during the period corresponding to the middle half of light phase in the nursing period, irrespective of whether the nursing mother was natural or foster. The free-running periods, in the blinded pups tested, were fairly close, majority ranging between 24.29 and 24.46 h. Extrapolation of free-running rhythm suggested that free-run of the rhyhtm started around 2-3 weeks of age. These findings indicate that the endogenous rhythm is entrained by the mother vir nursing the pups during the nursing period.

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CRITICAL PERIOD FOR THE ENTRAINMENT OF THE CIRCADIAN RHTYHM OF BLINDED PUPS BY THE FOSTER MOTHER RAT. SASAKI, Y., MURAKAMI, N., AND TAKAHASHI,K. Dept. of Medical Chemistry, Tokyo Metropolitan Institute for Neurosciences, Fuchu-city, Tokyo, 183

We have previously reported that the circadian rhythm of blinded pups, optically enucleated on day 1, was entrained by foster mother with a rhythm inverted to that of natural mothers. To determine how soon the mother-pup exchange should be done for the rhythm of blinded pups to be entrained by foster mother, we measured drinking rhythm of blinded pups raised under various nursing conditions. The drinking rhythm was determined once a week between 4 and 8 weeks of age by determination of water consumption every 4 h for 48 h. All pups transfered to foster mother before the 4th day of birth showed the same phase angle of drinking rhythm with that of the pups raised by natural mother under tha same lighting condition. On the other hand, the rhythm of the pups transfered after 10 days of age was not entrained by foster mother. The pups transfered between 5 and 7 days of age showed various rhythms, including rhythm influenced by both foaster and natural mothers. These results indicate that the critical period for the entrainment is between 5 and 10 days.

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TRANSPLANTATION OF THE SUPRACHIASMATIC NUCLEUS IN RATS SUBJECTED TO BILATERAL LESIONS OF THE SUPRACHIASMATIC NUCLEI. SAWAKI, Y. and KAWAMURA, H. Dept. of Neuroscience, Mitsubishi-Kasei Inst. of Life Sciences, Machida-shi, Tokyo 194

The suprachiasmatic nucleus (SCN) of the hypothalamus has been proposed as a circadian pacemaker in rodents. Electrolytic lesions of the bilateral SCN in Wistar strain male rats produced loss of circadian rhythmicity in wheel-running activity. In the SCN lesioned, arrhythmic rats, transplantation of the SCN was performed. Transplants were obtained from neonatal rats on the second day after birth. Coronal brain slice of about 1 mm thickness was prepared and each SCN was punched out with a stainless steel needle (0.96 mm I.D.). Pieces of hypothalamic tissue containing SCN were grafted with 30-40 µl Dulbecco solution into the third ventricle of the SCN lesioned rat under anesthesia. After transplantation, one month was allowed for recovery and wheel-running activity was again measured at least for one month.

In rats indicating recovery of apparent circadian rhythm in wheel-running activity under constant dark condition, survival of the transplant was observed. Histological examination revealed ingrowth of the tissue into the periventricular zone of the hypothalamus. These findings suggest a possibility of reinnervation of the grafted SCN as a circadian oscillator into SCN lesioned arrhythmic rats, which resulted in recovery of the circadian rhythmicity.

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CIRCADIAN RHYTHM OF ADENOSINE 3',5'-MONOPHOSPHATE CONTENT IN SUPRACHIASMATIC NUCLEUS AND VENTROMEDIAL HYPOTHALAMUS IN THE RAT. MURAKAMI, N. and TAKAHASHI, K. Dep. of Medical Chemistry, Tokyo Metropolitan Institute for Neurosciences, Fuchu-city Tokyo.

Twenty-four hour patterns of adenosine 3',5'-monophosphate (cAMP) contents in suprachiasmatic nucleus (SCN) and ventromedial hypothalamus (VMH) were determined at 3 h intervals under various conditions in adult male rats. The cAMP levels in both the nuclei showed a circadian variation with a clear peak time, which shifted under constant conditions (blinded and constant illumination). Under both entrained and free-running conditions, a consistent phase angle difference of 9 h in peak time was observed between cAMP rhythms in the SCN and VMH. The cAMP rhythm in the VMH synchronized with a restricted feeding time, when the rats were subjected to restriction of access to food under constant light. On the contrary, that in SCN did not synchronize with the restricted feeding time.

These results indicate that the 24-h patterns of cAMP contents in SCN and VMH are the manifestation of endogeneous circadian rhythms. However, the generating mechanism of cAMP rhythm may be different between SCN and VMH, as the restricted feeding regime affected the cAMP patterns of both the nuclei in different ways.

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CIRCADIAN RHYTHMS IN ELDERLY PERSONS IN VILCABAMBA, ECUADOR. OKUDAIRA, N., OHTANI, K.* AND TORII, S. Dept. of Physiology, Toho University School of Medicine, Ohta, Tokyo 143.

To investigate the circadian rhythms in healthy elderly persons living without institutionalization, three male and three female volunteers, ages 85-94 (mean age=91.2 years, weight 55.2 kg, and height 146.8 cm), in Vilcabamba, Ecuador were each observed for 5 days during July 1981. Oral body temperature (BT), radial pulse rate, and blood pressure (BP) were measured every four hours during waking hours. In addition, a daily sleep log was collected in which the subjects indicated all sleep periods. They followed their normal daily routine; working out in the fields, doing housework, etc. The sun rose at 6:00 and set at 18:00 during the time these measurements were taken. Data was analyzed using inferential statistical method assigning a 24-hour cosine to each time series to determine the acrophase, mesor, and amplitude of each rhythm.

Acrophase detection among the measurements for BT, diastolic BP and sleep-wake cycle occurred at -232.6°, -294.4°, and -22.6° respectively and was statistically significant. There were no significant differences in the internal acrophase (referred to mid-sleep) for BT between the elderly in Vilcabamba and Kanabrocki's young soldiers (Kanabrocki et al, 1973). This finding plus our REM sleep data of 20 elderly persons in Vilcabamba strongly suggests that no change occurrs in the "strong oscilator" in the elderly in Vilcabamba, Ecuador.

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RESPONSES OF HIPPOCAMPAL NEURONS TO CONDITIONED DISCRIMINATIVE STIMULUS. SAKAMOTO, M., *OGURI, M. and TORII, S. Dept. of Physiol., Sch. of Med., Toho Univ., Otaku, Tokyo, *Fac. of Sci., Toho Univ., Narashino, Chiba.

Ninety six single neurons were recorded from CA1 fields of the dorsal hippocampus in the freely moving rat and were studied in relation to behavior of the rat. The rat on the movable testing platform was presented pure tones, flash light and other natural stimuli. We found ten neurons which fired maximally when the rat positioned in particular parts of the experimental room. They were similar to hippocampal neurons previously reported as "place unit". However discharges of these neurons were not solely dependent on position of the rat. In most cases maximal responses required rat's spatial orientation, which implied various unidentified specific sensory inputs. For example covering of the rat's eye by the experimenter's hand produced vigorous, prolonged discharges in one neuron only when the rat was in a particular part of the experimental room, not in other parts of it. Conditioned tone stimulus which was previously associated with rewards produced the same effects.

These results suggesst that activation of place units depend on multimodal sensory inputs each of which is particularly significant for the rat.

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LATERAL HYPOTHALAMIC UNIT ACTIVITY AND FEEDING BEHAVIOR IN FREELY MOVING RATS. Muramoto, K-I., Sasaki, K., Ono, T., Nishino, H. and Fukuda, M. Department of Physiology, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Sugitani, Toyama 930-01.

It has been repeatedly demonstrated that the lateral hypothalamic area (LHA, feeding center) is important in the control of feeding. Only a few electrophysiological studies, however, have investigated relations between LHA unit activity and real feeding behavior. To study such relations, single unit activity was recorded from the LHA of freely moving rats during ad lib. feeding, during feeding after food deprivation, and after intraventricular injection of norepinephrine (NE) in satiated rats. Of 122 neurons tested during ad lib. feeding, firing rate changed (usually inhibitory) in 52% during access to food, picking up with tongue, and chewing. In one fourth of 47 neurons tested, activity depression during feeding episodes was greatest immediately after food deprivation and least during satiation. Spontaneous unit activity was independent of deprivation duration. Of 47 neurons tested during intraventricular NE-induced feeding, spontaneous unit activity decreased in 26% and increased in 6%. Results suggest that LHA neurons are involved in ad lib. and intraventricular NE-induced feeding behavior, and the unit activity of some is modified by the motivational state such as hunger or satiation.

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THE INNERVATION PATTERN OF AFFERENT TO THE SYRINX IN PIGEONS.

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All avian species are classified into two categories of the song ontogeny (canary type and pigeon type). Two categoric type of song ontogeny related clearly to the structure of a pair of halves of syrinx and the neural innervation patterns of their efferents. One type (canary) gives symmetric pattern of the innervation while the other one (pigeon) does asymmetric pattern for a pair of the halves.

However, afferent neurons (vagal ganglion cells) innervate in different pattern from that of the efferent. The afferent innervation of vagal ganglion is symmetry. By the HRP method injected to either half of syrinx, the afferent neurons innervate dominantly an ipsilateral half of either syrinx.

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SUBCELLULAR DISTRIBUTION OF GLUTAMINE SYNTHETASE IN RAT BRAIN. KANAMATSU, T. and HIRANO, S. 2nd Dept. of Physiology, Toho Univ. Sch. of Med., Ohmori, Tokyo 143

Glutamine synthetase (GS) catalyzes the ATP-dependent formation of glutamine from glutamate and ammonia. In the brain, glutamine formation by GS could provide a means for detoxifying ammonia and could play an important role related to the metabolism of glutamate which is one of excitatory neurotransmitters. We attempted to investigate both the subcellular distribution and cellular localization of GS. When the brain homogenate was used as the enzyme source of GS, non-linear mesured rates of GS could observed. The factors disturbing linear mesured rates contained in the precipitate by centrifugating a brain homogenate suspended in 10mM imidazol buffer for 20 min. at 27000g. And this disturbing effect could not observed by the addition of 0.5% Triton to the assay medium. Accordingly, we used the assay medium containing 0.5% Triton for mesuring total GS activity in brain tissue. 37% of GS distributed in the supernatant by centrifuge 27000g, 20min., then 36% of GS was extracted by the buffer containg 100 mM K^+ ions to the supernatant. And GS in the ppt was 39% of the total GS. Easily extractive GS with ionic medium was mainly localized in both the nucleus and myelin-1 fractions. GS in the ppt, so called membrane-attached GS, was broadly distributed in sub cellular fractions, especially in the microsomal fraction. Cellular localization of GS in brain tissue was definitely identified in astroglia cells.

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STUDIES ON ENHANCED UPTAKE OF PLASMA TRYPTOPHAN INTO BRAIN FOLLOWING ADMINISTRATION OF GLUCAGON IN THE RAT. KOHSAKA, S., TAKAMATSU, K., YIN, W., and TSUKADA, Y. Department of Physiology, Keio University School of Medicine, Shinjuku, Tokyo 160.

A content of brain tryptophan was measured following an intramuscular injection of glucagon (5 mg/kg). The brain tryptophan level increased significantly at 30 and 60 min after the injection and it restored to the normal level by 120 min. The extent of the increased level of brain tryptophan depended on the injection dose of glucagon (1-10 mg/kg). However, the plasma tryptophan levels remained unchang even after the injection of high dose of glucagon (10 mg/kg). The contents of 5-HT and 5-HIAA in the brain also increased significantly following the glucagon injection. A radioactive tryptophan was injected intravenously and the uptake of tryptophan into the brain was investigated. The ratio of brain tryptophan radioactivity to the plasma tryptophan radioactivity increased significantly following the injection of glucagon. The contents of brain amino acids were measured at 60 min after the injection of glucagon. The levels of tryptophan and valine significantly increased, however, no significant changes were detected in the contents of other amino acids in the brain.

From these results, it was speculated that the glucagon stimulates the uptake of tryptophan into the brain in a specific manner.

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IMMUNOHISTOCHEMICAL DEMONSTRATION OF INTRINSIC INTERNEURONS MEDIATING CORTICALLY INDUCED IPSPS IN CAT RED NUCLEUS. MURAKAMI, F., KATSUMARU, H. AND TSUKAHARA, N. Lab. of Higher Nervous System, National Inst. Physiol. Sci., Myodaiji, Okazaki 444

We have demonstrated the existence of GABAergic interneurons within red nucleus by an immunohistochemical study combined with degeneration methods and intracellular injection of HRP. Fixed tissues containing red nucleus were processed for enzyme immunohistochemistry utilizing antibody to GAD (glutamate decarboxylase, a generous gift from Dr. J.-Y. Wu). GAD positive small (10-15 μm dia.) neurons were found throughout the nucleus. In cats whose sensori-motor cortex was ablated before immunohistochemical procedure, degenerating axon terminals were found on GAD positive neurons under the electron microscope. On the other hand GAD positive terminals were found to synapse on rubrospinal neurons identified with the aid of HRP intracellular injection. Since the morphology of these GAD positive neurons in the red nucleus is similar to the intrinsic interneurons demonstrated by Golgi studies, it was concluded that these GAD positive neurons are the interneurons mediating IPSPs in rubrospinal neurons induced by stimulation of sensori-motor cortex.

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On the appearance of regulatory factors for the function of muscarinic acetylcholine receptor in the central nervous system of postnatal developing rat. HONDA,K,*IKUI,H. and KAMIYA,H. Dept.of Pharmacology, School of Pharmaceutical Sciences, and *Dept.of Neurosurgery, School of Medicine, Fukuoka University, Fukuoka 814-01

We examined the properties of muscarinic acetylcholine receptor(mAChR) in the striatum of developing rat by the binding technique with 3H-quinuclidinyl benzilate(^{3}H-QNB) as a muscarinic ligand. From the displacemental experiments by oxotremorine(OXOT), a muscarinic agonist, of ^{3}H-QNB binding to the striatal membranes from adult rat, we observed a conversion of low affinity sites into high affinity sites for OXOT in the presence of 2 mM Mn^{2+}. However, this conversion by Mn^{2+} was little influenced during postnatal day 5 -20. The ability of guanine nucleotides to convert the higher affinity sites into low affinity sites was demonstrated by even 0-day-old rat. Furthermore, the decreasing effect of the affinity for OXOT was partially inhibited by 2 mM Mn^{2+} on the postnatal day 10. By the binding assay of ^{3}H-guanylylimidodiphosphate(^{3}H-Gpp(NH)p), the non-hydrolysable GTP analogue, the density of ^{3}H-Gpp(NH)p binding was observed in the highest in the striatal membranws of postnatal days 1--5. This density decreased and sustained the constant level after the postnatal day 10. The result suggested that striatal mAChR of developing rat might be matured by the postnatal day 10 by the above-mentioned factors.

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PROPERTIES OF INTRACELLULAR α-BUNGAROTOXIN BINDING COMPONENTS IN CULTURED SKELETAL MUSCLE CELLS.
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Three forms of intracellular α-bungarotoxin binding components were found in cultured skeletal muscle cells with sedimentation coefficients of 9S, 5S and 3.5S. All these intracellular components could bind d-tubocurarine or carbamylcholine in a competitive manner with α-neurotoxins, and cross-reacted with antisera against acetylcholine receptors from electric fish. When glycoprotein synthesis was inhibited at different stages of biosynthetic pathways by cycloheximide, tunicamycin or monensin, each of the three intracellular components was affected in a different manner or to a different extent, depending on the inhibitor used. These results, together with our unsuccessful attempts to convert the 9S form into the smaller forms, suggested that these three forms of intracellular components may represent acetylcholine receptor precursors at different stages of subunit assembly.

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FULL AUTOMATICAL DETERMINATION OF CATECHOLEAMINE, INDOLEAMINE AND THEIR PRECURSOR OR METABOLITE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. OHTANI, S., OHTANI, K. and HORI, S. Dept.Neurochem., Tokyo Metropolitan Institute for Neurosciences, Fuchu-city, Tokyo 183

The simultaneous determination of catecholeamine, indoleamine and their precursor or metabolite in biogenic materials such as brain tissue extract or cerebrospinal fluid is useful in both psychophysiological research and clinical diagnosis. Previously we reported the determination of tryptophan, 5-HT and 5-HIAA in brain tissue extract by high-performance liquid chromatography with a weak acidic cation exchange resin. In this report, we developed a full automatical system for determination of tyrosine, dopa, dopamine, norepinephrine, HVA, tryptophan, 5-HT and 5-HIAA. Applications of three different separation methods with a weak acidic cation exchange resin equilibrated by 0.5M citrate-sodium citrate(pH4)-20% methanol, a reversed phase resin(silica-ODS) equilibrated by 0.1M citrate-sodium citrate(pH3.6)-0.1M $HClO_4$-10% methanol and a reversed phase resin equilibrated by 0.02M citrate-sodium citrate(pH2.2)-3% propanol-0.2M $NaClO_4$-0.003% SDS were performed. Combinational use of these different separation methods is useful to make sure the identification and value of unknown materials.

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ABNORMALITIES OF CORTICAL NEURONS OF THE SNELL DWARF CEREBRUM. NOGUCHI, T., SEKIGUCHI, M.*, SUGISAKI, T.** and TSUKADA, Y.** Dept of physiol, Toho Univ Sch of Med, Ohmori, Tokyo-143, and Depts of Anat* and Physiol**, Keio Univ Sch of Med, Shinjuku, Tokyo-160

In the Snell dwarf (dw/dw) motor cortex, the cell number, the stratification of neurons and the portion of layer-widths were absolutely identical to those of the control (+/?). By using the Golgi-Cox method, however, the pyramidal neuron showed to have small perikarya, short primary dendrites with sparse branchings, and the scarce spine density on the dendrites. The corpus callosum of the dwarf was organized with less number of fibers than that of the controls, and the staining for myelin basic protein revealed a considerably reduced positive-fibers of radiation in this area. Contents of Thy-1 of the three parts of brain, the cerebrum, cerebellum and brain stem, were significantly lower than those of the controls, but the monoamine contents were comparable to those of the controls in the cerebrum and brain stem.

These results clearly indicate that the Snell dwarf cerebrum shows the retarded neuronal growth; a reduction in volume of neurons, an underdevelopment of axon and dendrite, and a retarded maturation of spine, in addition to the arrested glial proliferation. At present, however, it is still unclear which of hormone, GH or T_4, is an essential potentiator for the neuronal growth.

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STUDIES OF CRITICAL PERIOD TO REQUIRE GROWTH AND THYROID HORMONES ON RETARDED CEREBRAL MYELINOGENESIS OF SNELL DWARF MICE.

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We reported that the Snell dwarf cerebrum shows a poor myelination, and that administration of GH and T_4 to the dwarfs (dw/dw) during the first 40 days of postnatal life restored 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNPase) activity (marker for myelinogenesis) to the level of the normals (+/?), and was accompanied by normalization of the pattern of spontaneous locomotion activity.

In attempting to define the critical period to require the hormones for myelination, the administration period was divided into two phases; the first postnatal 20 days and the next 20 days of postnatal life. The dwarfs receiving the hormones during first 20 days or next 20 days of postnatal life gained their body weight. The hormone administration during the first 20 days resulted in distinct increase in brain weight, DNA content, CNPase activity and, in addition, spontaneous locomotion activity with a diurnal periodicity. However, the administration during the next 20 days did not restore the cerebral development by means of biochemical and behavioral indicators.

These results indicated that the critical period of hormone administration for cerebral development will be restricted between the day of birth and the 20th days of age.

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EFFECTS OF PROTEASE INHIBITORS ON THE ACTIVITIES OF MYELIN ASSOCIATED ENZYMES IN BRAIN OF JIMPY MOUSE.

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In order to elucidate more clearly the disturbances of myelin formation in the central nervous system of mice with jimpy mutation, which is characterized by the spontaneous occurrence of marked tremor, unsteady gait and generalized tonic-clonic seizures at the time of myelination, we investigated the developmental changes of CNP and CEH activities in the myelin fraction from brain of jimpy mice. The effects of leupeptin or bestatin, which are protease inhibitors known to suppress breakdown of a myelin acidic protein, on the activities of CNP and CEH in the developing and maturing central nervous system of the mutant are also studied. The results obtained was summarized as follows; 1. The reduced activities of total CNP and CEH in the central nervous system of jimpy mice seems to be proportional to that of a reduced amount of total myelin rather than a specific defect of the enzyme protein synthesis. 2. Continuous administration of leupeptin or bestatin at early postnatal stage resulted in a striking recovery of myelin CNP and CEH activities in the central nervous system of jimpy mice, but not resulted in a prolongation of their life span.

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EFFECTS OF AXOTOMY, DENERVATION AND NEURAL ACTIVITY ON NERVOUS SYSTEM-SPECIFIC PROTEINS IN THE SUPERIOR CERVICAL SYMPATHETIC GANGLION OF THE RAT. ANDO, M., MIWA, M., NAGATA, Y. and KATO, K.* Dept. of Physiol., Sch. of Med., Fujita-Gakuen Univ., Toyoake, Aichi 470-11 and *Dept. of Biochem., Inst. for Develop. Res., Aichi Prefec. Colony, Kasugai, Aichi 480-03

Among enolase isozymes in the superior cervical ganglion (SCG) of the rat, neuron-specific $\gamma\gamma$-enolase decreased rapidly after denervation and stayed at low level for two weeks, while the isozyme remained unchanged after axotomy. On the contrary, ganglionic non-neuronal $\alpha\alpha$-enolase and $\alpha\gamma$-hybrid form increased remarkably to reach a maximum at the second day after denervation. Both denervation and axotomy caused a large increase in the ganglionic S-100 protein, an astrocyte-specific protein, during the first week after the operations, while the protein content decreased after two weeks of their denervation or axotomy. The amounts of three forms of enolase isozymes and S-100 protein in the excised SCG were not altered by pre- or post-ganglionic electrical stimulation, by addition of ACh, or by high K^+ in the incubation medium for 3 hours. But, addition of catecholamines, such as norepinephrine or dopamine, produced a clear increase of $\alpha\alpha$- and $\alpha\gamma$-enolase isozymes and S-100 protein, but not of $\gamma\gamma$-enolase in the SCG.

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EXTRACTION AND CHARACTERIZATION OF THE BIOLOGICALLY ACTIVE SUBSTANCES FROM SEA URCHINS IN FIJI. KIHARA,H., ANRAKU,M. AND HASHIMURA,S. Dept. of Physiol., Fac. of Med., Kagoshima Univ., Kagoshima 890

The lyophilized spines of sea urchins collected in Fiji were extracted twice with 70% EtOH and followed by chromatography with Amberlite IR-120B. The extract completely hemolyzed rabbit erythrocytes in one hour at 120-fold dilution of the stock solution. The extract posseses the lethal activity killing the majority of small fishes tested at 100 fold dilution of the stock solution. The completely recoverable nervous conduction block took place by the extract. The extract increased the frequency of miniature endplate potential (MEPP) immediately and significantly without effect on the amplitude and time course. The increase of MEPP frequency by the extract occured even in the solution containing excess K but no Ca. Addition of Mg or Mn caused the further increase of MEPP frequency in the same conditions. These results suggested that the extract increased the permeability of the nerve terminals to divalent cations such as Mg or Mn.

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INACTIVATION OF CORTICOTHALAMIC PROJECTION BY COOLING OF THE RAT VISUAL CORTEX. KAYAMA, Y. and SHOSAKU, A. Dept. of Neurophysiology, Inst. of Higher Nervous Activity, Osaka Univ. Medical School, Kita-ku, Osaka 530

Unit discharges were recorded from the dorsal lateral geniculate nucleus (LGN) and from the visual part of the thalamic reticular nucleus (v-TR) of urethane-anesthetized rats, with and without inactivation of the visual cortex (VC) by flowing a cold saline on the cortical surface. While VC was cooled, substantially all v-TR neurons were depressed. This supports the view that the corticothalamic projection exerts a tonic excitatory effect upon target neurons; its blocking may bring the latters into a depressed state (disfacilitation). Although LGN was expected to receive the same effect of cortical cooling with the same strength as observed in v-TR, the incidence of neuronal depression in LGN did not exceed 40%. This is probably because LGN neurons receive a tonic inhibition from v-TR besides a tonic excitation from the visual cortex. Cortical cooling provides LGN neurons not only with disfacilitation but also with disinhibition resulting from disfacilitation of v-TR neurons. This would make the effect of cortical cooling less clear in LGN.

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PROPERTIES OF VISUAL CORTICAL NEURONS COMPOSING THE CORTICO-CLAUSTRAL LOOP CIRCUIT. TSUMOTO, T. and SATO, H., Dept. of Physiol., Kanazawa University Medical School, Takaramachi, Kanazawa 920

Effects of electrical stimulation of the dorsocaudal sector of the claustrum (CLdc) on activities of 150 striate cortical neurons were observed in cats anesthetized with N_2O and Nembutal. About one-fourth of the cells were excited with latencies of 5-20 msec and another one-fourth were inhibited with onset latencies of 17-83 msec. Almost all the cells with special complex properties, which were located in layer V of the cortex, were excited by CLdc stimulation, while 75 % of the hypercomplex cells in layer II+III were inhibited. About half of the complex cells were excited and 30 % of the simple cells were inhibited by the stimulation. The other simple cells and all the cells with exclusively ON or OFF receptive field, which were located in layer IV, did not receive any effect from the CLdc. Most of the cells projecting to the dorsal lateral geniculate nucleus were inhibited by the stimulation. Several cells were activated antidromically by CLdc stimulation. All of these cortico-claustral cells were of simple type and located in layer VI. Since cells in layers II+III and V project to other cortical areas and the mesodiencephalon, respectively, the present results suggest that the claustro-cortical projection may selectively control efferent projection systems from the visual cortex.

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FUNCTIONAL ARCHITECTURE IN CLARE-BISHOP CORTEX OF THE CAT. TOYAMA, K., HASHIMOTO, S. and FUJII, K. Dept. Physiol., Kyoto Prefectural Med. School, Kawaramachi, Kyoto 602.

Neuronal responses to movement of visual stimuli in three-dimensional space were studied along a track perpendicular to columnar structures in the Clear-Bishop (CB) cortex of the cat. CB neurons were classified into 4 functional groups: 1) approaching (AP) cells responding to approaching movement, 2) departing (DP) cell responsive to departing movement, 3) equi-distance (ED) cell to front-parallel movement and 4) nonselective (NS) cells to all types of movements. Each group of cells appeared in a band of 0.3-0.6 mm across, suggesting existence of columnar structures concerning responsiveness to three-dimensional movement. Similar study demonstrated columnar structures of about same size for ocular dominance, responsiveness to movement disparity and that to a change in stimulus size, and smaller (5 μm/degree) columnar structures for directional preference.

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RESTORATION OF NEURONAL PLASTICITY IN CAT VISUAL CORTEX BY LOCUS COERULEUS STIMULATION.
WATABE, K., KASAMATSU, T.*, SCHÖLLER, E.** and HEGGELUND, P.** Dept. Physiol., Aichi-Gakuin Univ. Dent. Sch., Nagoya 464; Div. Biol., Calif. Inst. Tech., U.S.A.*; Neurobiol. Lab., Univ. Trondheim, Norway**

We have shown that norepinephrine (NE)-containing terminals in the visual cortex are necessary to maintain and enhance visual cortical plasticity. In the present study, we have examined whether electrical stimulation of NE-containing cells in the locus coeruleus (LC) can restore neuronal plasticity in the visual cortex of aged kittens (n=4) and adult cats (n=4). Monocularly deprived animals kept in the darkroom were brought into a normally lit experimental room for 2hrs daily in consecutive 6 days. The LC was continuously stimulated during these periods of monocular visual experience. We could induce changes in the ocular dominance distribution in the visual cortex (Binocular cells; 41% of visually responsive cells), and this restored, visual plasticity lasted for at least 3 weeks after cessation of the stimulation.

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EYE MOVEMENTS EVOKED BY ELECTRICAL STIMULATION OF THE FRONTAL CORTEX IN THE CORONARY SULCUS OF THE CAT.
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The frontal cortex concerned with eye movements was investigated by electrical stimulation in the cat. Pulse train stimulation of lateral part of the frontal cortex at the coronary sulcus evoked rapid medial movement of the contralateral eye, and slow lateral movement of the ipsilateral eye. If the stimulus intensity was increased an withdrowal of the eye ball or centering eye movement was observed. The latency was as short as about 19 msec. The latency of eye movement following cortical stimulation was found to be shorter in the coronary sulcus than in the medial wall of the hemisphere under the curuciate sulcus or in the presylvian sulcus. There are no conjugate eye movements after lesion of this cortex in the coronary sulcus. The affected eye can't follow in either direction, especially medial movement from the central eye position. The conjugate movement is almost recovered after one day but the disturbance of medial movement of the affected eye remained over one day.

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MONOCULAR OPTOKINETIC NYSTAGMUS IN CATS TO TEXTURED VISUAL PATTERN
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Horizontal optokinetic nystagmus(OKN) to various textured visual patterns to one or both eyes was studied in unanaesthetized cats. In monocular OKN, motion of the textures of random noise or checkerboard elicited larger slow phase velocities than stripes with regulary or randomly arranged vertical bars when the stimulus was moved in temporonasal direction at a velocity above 10 deg/s and in nasotemporal direction. To temporonasal stimulation at a velocity below 10 deg/s, there were no or little differences in slow phase velocities due to textures. Binocular OKN showed the similar preference for the textures as temporonasal OKN.

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BILATERAL ACCOMMODATION RESPONSES AND CONVERGENCE CHANGES IN HUMAN SUBJECTS.
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The amount of accommodation in the bilateral eyes was estimated to be equal from the clinical observations, but it was never measured because of the difficulties in simultaneous recordings of the refractive power changes in bilateral eyes. In order to decide whether the estimated accommodation values were correct or not, two ratios, A/A (accommodation in the right eye/ acc. left eye) and AC/A (accommodative convergence/ accommodation) were measured with a new system consisting of two refractometers (Auto Ref, R-1, Canon Inc.,) and a microcomputer (Apple II). The measurement was carried out on 38 subjects (A/A) and 12 subjects (AC/A) of 9 to 31 years old. The A/A relation was linear, and the plotted line intersected the rectangular axes at 45°. The A/A relation was similar among emmetropes, hypermetropes, myopes and anisometropes. The ratio AC/A, was 2.85± 0.91 using an accommodation stimulator, and this value well coincided with the ones estimated previously. When the optical target was moved closer under natural view condition, the ratio increased up to 4.05±1.59 as the summation of the accommodative and proximal convergences.

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SPECRAL OFF RESPONSES OF OPPONENT COLOR CELLS IN THE LGN OF MACAQUE MONKEYS.
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The responses of opponent color cells such as the Red/Green(R/G)- and the Yellow/Blue(Y/B)-types were recorded in the lateral geniculate nucleus of macaque monkeys. Spectral sensitivities of the ON and the OFF responses of each cell to flashes of monochromatic light equated for energy were measured. And the following properties were found:the range of wavelengths where the +R-G cell showed the excitatory OFF responses was widely spread rather than that where the +G-R cell did the excitatory ON responses. With comparison between two sorts of Y/B cells(+Y-B and +B-Y), the range of wavelengths of excitatory OFF responses of +B-Y cells were extensive rather than that of the excitatory ON responses of +Y-B cells.

These results of spectral OFF responses in the macaque LGN cells agreed qualitatively with the facts that in the psychological experiments, a person with normal color vision could see complementary color in the after-images evoked on cessation of spectral stimulation and he could sense more reddish (or bluish) corresponding to spectral stimulus.

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RESPONSE PROPERTIES OF VENTRAL LATERAL GENICULATE NUCLEUS CELLS IN THE CAT.
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Response properties of ventral lateral geniculate nucleus(LGV) cells were examined in the cat anesthetized with N_2O/O_2. LGV cells responded with single spikes to stimulation of optic chiasm(median, 3.8 ms, n=132). The postexcitatory inhibition was only short-lasting and the rebound discharge was not elicited. Optic nerve fibers innervating LGV cells had slower conduction velocities(median,8.5m/s, n=22). Among 235 LGV cells tested, 120 cells responded antidromically(n=40) or synaptically(n=80) to stimulation of the superior colliculus. The majority(83/101) of cells responded predominantly to visual stimulation of the contralateral eye. The sizes of receptive-field center of LGV cells had significantly larger than those of dorsal lateral geniculate cells(15° vs 4°, $p<0.001$). The receptive-field center properties of LGV cells(n=86) consisted of ON-phasic(29), ON-OFF(19), ON-tonic(14), movement-sensitive(12), ON-OFF-inhibited(5), ON-inhibited(5) and OFF-phasic(2). Distribution of receptive-field centers revealed a centro-peripheral gradient of retinal representation within the LGV. A visuotopic representation was seen in the LGV. The lower visual field was represented anteriorly in the nucleus and upper one posteriorly. The medial part of the LGV represented the nasal field while the lateral part did the temporal periphery.

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THE EFFECTS OF KAINIC ACID LESIONS IN THE RAT VENTRAL LATERAL GENICULATE NUCLEUS ON VISUOMOTOR FUNCTION. NAGATA, T., MATSUMO, K. and HAYASHI, Y. Dept. Physiol. and Ophthalmol., Hyogo College of Medicine, Mukogawa-cho, Nishinomiya, Hyogo 663.

Effects of kainic acid (KA) lesions in the rat ventral lateral geniculate nucleus (LGv) on visuomotor function were examined. Visuomotor function was assested by the pupillary reflex (PR), the optokinetic nystagmus (OKN) and the vestibulo-ocular reflex (VOR). The optokinetic nystagmus was produced by 4° wide vertically oriented black and white stripes rotated sinusoidally at 0.014 Hz in horizontal direction with the amplitude of 110°. Contrast between black and white stripes was varied from 0.8 to 2.0 log unit. In intact rats, OKN was observed clearly still at the contrast of 0.8 log unit but it was not seen even at the contrast of 2.0 log unit in LGv-lesioned rats. Especially, the slow phase of OKN and tracking eye movements to the sinusoidal stripe movements were completely abolished. No obvious difference was detected between intact and LGv-lesioned rats in PR and VOR. These results suggest that LGv is profoundly related to a manifestation of OKN rather than PR and VOR.

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MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED GANGLION CELLS (Y, X and W-CELLS) IN THE CAT RETINA. FUKUDA, Y. and HSIAO, C.-F. Dept. of Neurophysiol., Inst. of Higher Nervous Activity, Osaka University Medical School, Kita-ku, Osaka 530

1) Under anesthesia with N_2O/O_2 gass mixture single unit activities of retinal ganglion cells were recorded with HRP-containing microelectrodes. According to physiological properties they were identified as one of the three types, Y, X and W-cells. After current injections they were reacted with DAB to study their morphology at light microscopic level. All Y cells recovered (N=10) and all X cells (N=7) corresponded to α and β cells of Boycott and Wässle, respectively. W-cells (N=4) consisted of their γ and δ cells as well as unilateral horizontal broad range cell of Schkolnik-Yarros. 2) After HRP injections with coarse glass microelectrodes into the ganglion cell layer labelled ganglion cells were identified as one of the three types according to the criteria established above. Representative cells of the three types were selected and processed for electron microscop . On photomicrographs of serial sections contact areas of bipolar and amacrine synapses were measured by using an image analysing system. Covering ratio of bipolar to amacrine synapses was 0.6 in a W cell while it ranged from 1.2 to 1.9 in 2 Y and 2 X cells.

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RESPONSES AND MORPHOLOGIES OF TWO-TYPES OF HORIZONTAL CELLS OF THE CAT RETINA. SAITO, H. NHK Broadcasting Science Research Labs., Kinuta, Setagaya-ku, Tokyo 157

Response properties and morphological characteristics of two-types of horizontal cells of superfused cat retinal preparations were studied by intracellular recording and staining techniques using microelectrodes filled with Lucifer yellow CH. Both type A (axon-less) and type B (axon-bearing) horizontal cells showed hyperpolarizing responses to a spot of light. However, signal summation area of type A cells was larger than that of type B cells.

When Lucifer dye was injected into a single type A cell, a large number of the same type of horizontal cells were stained with gradually decreasing dye density with increasing the distance from the injected cell, exhibiting regularly spaced lattice-arrangement. Cells further than 0.8 mm from the injected cell were clearly visible. The cell density was 120-160/mm^2. Thus we conclude that all type A cells are connected side by side through tight junctions through which the dye can diffuse into neighbouring cells. This structural feature might be suitable for detecting the mean luminance level of the retina and controlling the receptor sensitivity through a negative feedback. In contrast, type B cells were stained always singly. Dendrites of type B cells extend radially in a circular area (80-100 μm ϕ) which is narrower than that of the single type A cell (asymmetric in shape, long axis 160-180 μm).

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ANALYSIS OF DIRECTIONAL SELECTIVITY IN GANGLION CELLS OF FROG RETINA. WATANABE, S.-I. AND MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo.

Many retinal ganglion cells of the frog, Rana catesbeiana, not only responded to a stationary, flashing light stimulus, but also showed directional selectivity to a moving stimulus crossing their receptive fields at proper speed to individual cells. In order to analyze synaptic mechanisms of the selectivity, intracellular recordings were made, when necessary, under effects of tetrodotoxin. The toxin eliminated spike discharges and enabled us to observe full amplitudes of EPSP's above the threshold of spike generation. In a toxin-containing solution, ON- and OFF-type cells responded to the moving stimulus solely with EPSP's; those evoked in preferred direction were much larger than those in null direction. In ON-OFF-type cells, polarization of cell membrane by an extrinsic D. C. current clearly demonstrated that EPSP's appeared prior to IPSP's in preferred direction. In null direction, on the contrary, IPSP's preceded EPSP's, thus suppressing spike generation. These observations suggested that the synaptic mechanisms of the directional selectivity in ON- and OFF-type cells are different from those in ON-OFF-type cells.

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GANGLION CELL RESPONSES TO WINDMILL PATTERN STIMULUS IN THE CARP RETINA. SUGAWARA, K. Dept. of Neurophysiol., Neuroinformation Res. Inst., Sch. Med. Univ. Kanazawa, Kanazawa 920

Lateral interactions were studied on ganglion cells of isolated carp retinas using a truncated windmill (TW; 8 vanes of 2 mm i.d. and 3.5 mm o.d.). Flashes of steady TW increased or decreased ganglion cell discharges recorded at the TW center. Rotation of the TW pattern further altered the discharge rate of some cells. These responses were classified into 4 types; 1, enhanced by steady TW but suppressed by rotation; 2, suppressed by both conditions; 3, enhanced by both; and 4, suppressed by steady but enhanced by rotation. Mean spreads of TW rotation slower than 0.1 mm/sec were effective to suppress discharges of type 1 and 2 cells, while those faster than 3.5 mm/sec were required to enhance discharges of type 3 and 4 cells. The responses of color-opponent cells to TW rotation were independent from wavelengths. In all the cells examined, central responses to small spots were partially masked by discharges induced by TW rotation. All the phasic (ON-OFF) type amacrines examined thus far responded with depolarization to TW rotation. The present results suggest that ganglion cells receive excitatory or inhibitory inputs post- or/and pre-synaptically from phasic and tonic type amacrines in the receptive field periphery.

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RESPONSES OF RETINAL AMACRINE CELLS TO TRANSRETINAL CURRENT STIMULATION. FUJIMOTO, M. and TOYODA, J. Dept. of Physiology, St. Marianna Univ. Sch. of Med., Miyamae-ku, Kawasaki 213

Transretinal current from receptor side to vitreous side depolarizes axon terminals of retinal neurons and facilitates the transmitter release. The response of amacrine cells of the carp retina to such current is rather complex because it stimulates the release of transmitters from both photoreceptor and bipolar cell terminals. After blocking receptor-bipolar transmission by adding glutamate in the perfusing media, the current elicited a depolarizing response in any type of amacrine cells irrespective of whether they are 'on' type, 'off' type or 'on-off' type. The depolarizing response is accompanied by a decrease in membrane resistance. The results indicate that the synaptic transmission from bipolar to amacrine cells is excitatory. These findings are consistent with the hypothesis that 'on' amacrine cells receive inputs from 'on' bipolar cells, 'off' amacrine cells from 'off' bipolar cells and 'on-off' cells from both 'on' and 'off' bipolar cells.

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EYE MOVEMENTS AND VISUO-VESTIBULAR INTERACTIONS DURING THE DORSAL LIGHT TILT REACTION (DLR) IN CARP. MITARAI, G., MORI, S., TAKABAYASHI, A. and TAKAGI, S. Dept. of Aerospace Physiol., Res. Inst. Environ. Med., Nagoya Univ., Chikusa-ku, Nagoya 464.

Lateral illumination induces a body-axis tilting reaction (DLR), up to 25 degrees maximally in the carp. The reaction was only detectable with a latency of 0.5 sec and the maximal tilt attained in 3-5 sec after the onset. Based on the following observations, it was concluded that such slowness of the reaction might reflect strong interactions in the posture control center: (1) During DLR, the eye position of the non-illuminated side followed the vestibulo-ocular response, but it was modified in the side illuminated, and (2) labyrinthectomy made the tilting perfectly visually-dependent (a 90° tilt for the lateral light), but the tilting speed was remarkably facilitated and the eyes moved vertically as if it were leading the tilting, indicating the loss of counteracting factor. The facts that the optic-lobe ablation did not affect DLR and the cerebellar responses elicited by the optic-disk and the eighth-nerve stimulations interacted each other, suggest that the cerebellum (corpus cerebelli) might largely contribute to the DLR control.

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ELECTRICAL MEMBRANE PROPERTIES OF OFF-CENTER BIPOLAR CELLS IN THE CARP RETINA. SAITO,T.AND KUJIRAOKA,T. Dept.Physiol.St.Marianna Univ Sch.of Med.

Electrical membrane properties were studied on off-center bipolar cells in carp retina by injecting either a ramp or a steady current through one barrel of a double-barreled electrode and recording the voltage drop through the other barrel. The current-voltage curve showed inward and outward rectifications which modified the amplitude of the light-evoked responses. The degree of membrane rectifications varied considerably from one cell to another. Both center and surround responses always reversed their polarity at a positive membrane potential level.

Off-center bipolar cells were stained by iontophoretic injection of Lucifer yellow dye after studying their electrical properties. The cells which were characterized by their small cell bodies lying close to the proximal part of the inner nuclear layer and their thin apical dendrites tended to show rectifying membrane properties, compared to those characterized by their large cell bodies lying close to the distal part of the inner nuclear layer and their thin apical dendrites. The results suggest that the cell body is the most probable site showing rectifying membrane properties and the variation of the membrane rectification may be related with the difference in the cell morphology.

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GABA RECEPTORS ON THE AXON TERMINAL OF BIPOLAR CELLS IN THE CARP RETINA. KONDO, H. and TOYODA, J. Dept. of Physiology, St. Marianna University School of Medicine, Miyamae-ku, Kawasaki 213

Effects of GABA and glycine applied electrophoretically were examined on 'on'- and 'off'-center bipolar cells in the carp retina. When applied at the outer plexiform layer, GABA produced a small depolarization in on-center cells, but a small hyperpolarization in off-center cells. Since these responses were blocked by cobalt ions, these effects is likely to be mediated by presynaptic, probably photoreceptor cells. When applied at the inner plexiform layer, GABA produced a hyperpolarization in on-center cells but no response in off-center cells. The hyperpolarizing response was resistant to cobalt ions, suggesting that the substance acted directly on the axon terminal of the on-center cells. During the response the cell's membrane resistance decreased. Thus GABA is very likely the inhibitory neurotransmitter from some amacrine cells to on-center bipolar cells. Glycine, when applied in either layer, did not have a noticeable effect on either type of bipolar cells. These cells seem to have no glycinergic inputs.

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COLOR OPPONENT BIPOLAR CELLS IN THE CARP RETINA. SAKAKIBARA, M. AND MITARAI, G. Dept. of Aerospace Physiol., Res. Inst. Environ. Med., Nagoya Univ., Chikusa-ku, Nagoya 464

Our previous results showed that the antagonistic receptive-field organizations of color-opponent bipolar cells in the carp retina could be divided into 4 types. To further their detailed organizations, the relation between response amplitude and size of stimulus light spot (ARC) was analysed by using several monochromatic lights. In the cells classified into the spatially segregated type, whose spectral-response peaks dissociate from each other between the center and surround, it was suggested that the receptive field consists of only red and green inputs since the each spectral peak could be identified when a small 0.1 mm spot was used and further confirmed by a monochromatic adaptation method. The difference in ARCs between red and green light stimulation revealed that the peak spectrum could vary with the size of spot used. The surround type, which shows color opponency only in the surround, was characterized by a large summative area to one color and a distinctive spatial opponency to the other. The center type, showed color opponency in the exclusively small center area less than 0.1 mm in diameter, suggested that the interactions between different color type cones contribute to the mechanisms rather than the influences from horizontal cells.

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CHANNEL CATFISH BIPOLAR CELLS. HIDAKA, S.* and NAKA, K.-I. National Institute for Basic Biology, Okazaki 444

Famiglietti et al. (1977) found in fish that the on- and off-center bipolar cells had their axonal terminals at the sublamina b and a. We have Golgi-silver impregnated bipolar cells in channel catfish, Ictalurus punctatus, and correlated the level of their axon terminal with the disposition of their dendritic arborization in the receptor terminals. Observations were made with both transmission and high-voltage electronmicroscopes. The sublamina-a (off-center) cells made three kinds of invagination into the receptor terminal; 1) close contact with the ribbons, 2) distal contact with both the horizontal cell distal processes and receptor terminals, and 3) a shallow invagination. The sublamina-b (on-center) cells lack the contact of the first kind. The catfish bipolar cells we have analyzed so far had their inputs from both the rods and cones. Results we present here suggest that morphological pathways for the receptor to bipolar signal transmission is more complex than we had expected.

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NONLINEAR DYNAMICS IN INNER LAYER OF CATFISH RETINA.
SAKURANAGA, M. and NAKA, K-I.*, Dept. of Physiol., Nippon Medical School, Bunkyo-ku, Tokyo 113, *National Institute for Basic Biology, Okazaki 444.

White-noise modulated current was injected into the horizontal cells and resulting responses were recorded from the type-C and -N cells in the retina of channel catfish, Ictalurus punctatus. The responses were decomposed into the linear and nonlinear components in terms of the Wiener analysis. Characteristics of signal transmission in the inner retinal layer was disclosed: The linear component of the type-C cells' response was very small and showed a band-rejection character around a frequency of 10 Hz. The second-order kernel had a stereotypical pattern and reproduced the cells' response with a reasonable degree of accuracy. On the other hand, both linear and nonlinear components had comparable share in the type-N cells' response. The nonlinear components were sharp transients and oscillatory wavelets. In the dark type-N cells showed spontaneous oscillation around 10 Hz. This oscillation was different in frequency from the wavelet in the stimulus-induced response but formed a mirror image to the band rejection of the type-C cells' response. We already reported that the signal transmission within the outer retinal layer is essentially linear. We conclude that characteristic nonlinearities appear in the inner retinal layer and are intrinsic to the cell types in the layer.

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CENTER-SURROUND INTERACTION IN THE VERTEBRATE RETINAL RECEPTIVE FIELDS. NAKA, K.-I., SAKURANAGA, M., and CHAPPELL, R. L*. National Institute for Basic Biology, Okazaki 444, Nihon Medical School, Tokyo 113, JAPAN and Hunter College, New York 10021, USA.

Incremental sensitivity, as defined by the first order kernels, was measured in the inner nuclear layer neurons in the catfish, Ictalurus punctatus, and the red-eared turtle, Pseudemys scripta elegans, retinas. Test stimulus was a white-noise modulated spot of light. Presence of surround illumnation, stationary random grating, 1) enhanced the neurons' (receptive-field center) incremental sensitivity, 2) made the kernels faster and more differentiating, and 3) linearized the response. These changes were not related either with the classical excitation or inhibition but reflected the subtle and significant signal processing in the outer retinal layer. Steady surround was well as modulated one had similar effects on the center response.

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LIGHT-RESPONSIVENESS OF DIFFERENT RETINAL CELL TYPES DURING SPREADING DEPRESSION (SD). SHIMODA, Y. and *TOMITA, T. Dept. of Ophthalmol. & Visual Sci., Yale Univ. Sch. of Med., New Haven, CT 06510 (USA), Dept. of Physiol., Kyorin Univ. Sch. of Med., Mitaka 181, and *Dept. of Physiol., St. Marianna Univ. Sch. of Med., Kawasaki 213.

The isolated retina of the bullfrog, Rana catesbeiana, when kept in low Cl^- Ringer's for a while, started to give rise to SD-characteristic potential changes (SDPs) repeating at some 10 min intervals. Using a pair of electrodes with their tips set close to each other, SDPs were recorded from inside and outside a cell simultaneously. The paired electrodes, when connected differentially, allowed recording of the membrane potential change of the cell during SD, together with the response of the cell to flashes of dim white light applied over the retina at 5 sec intervals throughout experiment. The intracellular electrode contained Procion Yellow dye and served also for later morphological identification of the type of the cell. Receptor, horizontal, and bipolar cells thus identified continued to respond to light flashes throughout the SDP period, though their response amplitude decreased in proportion to the cell depolarization during SDP. In amacrine and ganglion cells, their response to light was completely lost during the peak time of SDP accompanied by strong depolarization. The last two cell types appeared to play an active role in the SD mechanism.

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LATERAL SPREAD OF PHOTOPIC S-POTENTIALS AND DYE COUPLING IN THE CARP RETINA. TERANISHI, T., NEGISHI, K. AND KATO. S. Dept. of Neurophysiol., Neuroinformation Res. Inst., Sch. Med. Univ. Kanazawa, Kanazawa 920

Since horizontal cells of the fish retina are electrically coupled via gap junctions in a given cellular layer, a dye Lucifer yellow injected ionophoretically into a cell normally diffuses to several neighboring cells. In the carp retina, the spectral responses, amplitude·area relation and spatial decrement with spot displacement were examined on a hyperpolarizing component of photopic S-potentials. Recorded horizontal cells were intracellularly marked by the dye. The spatial properties of S-potentials were compared with the dye diffusion areas of different response types. The spatial summation of S-potentials, revealed by enlarging or displacing spots, was found to be smaller at the soma level than at the axon terminal level of the L and RG type horizontal cells. At the soma level, the spatial summation was found to be in the order of L < RG < YRB type. Correspondingly, the dye diffusion area was narrower at the soma level than at the axon terminal level of the L and RG type horizontal cells, and it was in the order of L < RG < YRB type at the soma level. Therefore, a clear-cut correlation appears to exist between the electrical and dye coupling areas via gap junctions along the cone-connected horizontal cell layers.

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EFFECTS OF DOPAMINE AND BICUCULLINE ON L-TYPE HORIZONTAL CELLS IN THE CARP RETINA.
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Dopamine (DA; 10-20 μM), applied to the residual vitreous fluid beneath isolated carp retinas, increased the amplitude of L-type S-potentials in response to central light spots, decreased that to distant spots and restricted an intracellularly injected Lucifer yellow (LY) into single cell bodies. Such DA effects were mimicked by bicuculline (Bcc), amphetamine, dibutyryl cAMP or forskolin (each 20 μM). Prior deprivation of DA cells from the retinas with 6-hydroxydopamine resulted in the opposites; reduction of the central S-potentials, increase of the lateral spread of these potentials and dye diffusion to numerous neighboring cell bodies. In the retinas deprived of DA cells, however, the application of DA, dibutyryl cAMP and folskolin produced the usual effects but Bcc and amphetamine had no effects on the horizontal cells. Further, the effects of DA, Bcc and amphetamine were prevented by haloperidol (20-40 μM) while those of dibutyryl cAMP and forskolin were not interfered with the dopaminergic blocker. The results indicate that in the carp retina Bcc acts on external horizontal cells indirectly through DA cells, which regulate the spatial properties of the cells by acting on their membrane and junctional resistance via a DA-receptor mediated mechanism.

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UPTAKE AND RELEASE OF ^{3}H-DOPAMINE IN THE RETINAL EFFERENT NERVE TERMINALS OF THE OCTOPUS, OCTOPUS OCELLATUS.
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The retinal surface of the isolated visual preparation of the octopus, consisted of the retina, optic nerve and attached optic lobe, was incubated in seawater containing 0.1 mCi ^{3}H-Dopamine (DA). Repetitive stimulation of the optic nerve during continuous perfusion of the retina with oxygenated seawater gave rise to an increase in radioactivity in perfusate. The result was much more clearer if outer segments had been thoroughly removed from photoreceptors, so as to expose the basement membrane to the bathing media : The effect of optic nerve stimulation appeared with much shorter latencies and the amount of increased radioactivity was greater with stronger stimulus intensities. The same result could be obtained from a simple preparation of isolated retinas or sliced preparations of the optic lobe : The preparation was contained in an acryl well and stimulating currents were applied across the tissue through a pair of silver wires placed in the well. The uptake and release of ^{3}H-DA was studied also in the reserpinized retina with the exactly same result. These results add further confirmation to our previous proposal that DA is a transmitter released from the efferent nerve of the octopus retina.

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AFTER-EFFECTS OF TRANSRETINAL CURRENT ON THE ERG.
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As reported previously the transretinal direct current applied locally on the bullfrog retina can modify the configuration of the ERG depending upon its intensity and its polarity, thereby the current leaves some effects on the ERG after switching-off. These after-effects are very definite in the eye cup preparation: a vitreous-negative current leaves, after cessation, a diminution of the ERG amplitude, while a vitreous-positive current makes ERG amplitude considerably large, which might be said a kind of rebound from supressive effects of the vitreous-positive current. In the isolated inverted preparation, however, it is very difficult to say if there exist such definite after-effects in this preparation as well. Amplitude and configuration changes are often encountered in the isolated inverted retina in the daily experiments, which might obscure some definite phenomena which otherwise could easily be found. This may be the main reason why any certain results have not been obtained so far for the isolated inverted retina. Anyway the differences between the phenomena seen in these two preparations might come for the most part from the presence and absence of the pigment epithelium.

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ELECTRIC RESISTANCE CHANGE OF THE CHICK RETINA IN THE COURSE OF DEVELOPMENT. SATO, T., SOMEI, K., MATSUMOTO, N., TAIRA, K., SUZUKI, T.A. and *MITA, T.(Prof. Emeritus) Dept. of Oral Physiol., Sch. of Dent. & *Dept. of Physiol.I, Sch. of Med., Iwate Med. Univ., Morioka 020

Following anesthetization with 1 mg *Nembutal*, 102 eyeballs were isolated from both the embryo and the chick aging from 14th day of incubation to 4th day of hatching. The retina was punched out from the posterior pole area of each eyeball onto a piece of rubber sheet. After removal of the sclera, it was set into the perfusing chamber, and both sides of its central part, 3.2 mm in diameter, were perfused independently with the artificial vitreous and choroidal humors warmed at 37°C and bubbled with a mixed gas of 97.5% O_2 and 2.5% CO_2. Electric resistance of the retina was measured every 5 minutes and the mean value of those measured was taken as a resistance value of each preparation. Five to 17 samples were collected for each daily age group.

During 14 and 16 days of incubation resistance of the retina decreased slightly but then increased markedly, from 1.2 to 1.7 KΩ, along the growth curve of the retina and of the oil droplet in the photoreceptor until the 1st day of hatching. Although most of the chicks raised in the dark remained in the high resistance group (1.9 KΩ), resistance of some chicks' raised under red light in day time decreased to the range between 0.9 and 1.7 KΩ and the occurrence rate increased from 40 to 82% with increase of the daily age. This would support our previous results obtained in vivo extraocularly.

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ON THE PII AND PIII SUBCOMPONENTS OF THE ISOLATED BULLFROG RETINA. KATSUDA, S. AND HANAWA, I. Dept. of Physiol., Kobe University, School of Medicine, Chuo-ku, Kobe 650.

The subcomponents of the PII and PIII responses of the isolated dark-adapted bullfrog retina were studied by the treatment with Ba^{2+} which has shown to reduce K^+ conductance of the cell membrane. The application of 0.05 mM Ba^{2+} on the vitreous side exhibited an inhibitory effects on the PII response, which were a reduction in amplitude and a rapid falling in the response as observed in the light-adapted retina. On exposing the vitreous side to the 2.0 mM Ba^{2+}, the PII response remarkably suppressed and only the negative-going potential change was observed. This negative response declined in amplitude by the treatment of 5.0 mM aspartate on the receptor side. As the stimulus intensity was raised, the amplitude of this PIII response increased, but the peak latency remained little changed. In the presence of Ba^{2+}, both the peak of the action spectrum of the PII and PIII responses shifted from 500 to 550 nm and restored to 500 nm by the following application of the 5.0 mM aspartate. These results suggest that the PII response consists of the fast and slow subcomponents which might reflect mainly cone and rod activities respectively, and that the PIII response of the 2.0 mM Ba^{2+}-treated retina consists of the proximal and distal responses.

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THE EFFECTS OF SEVERAL PERFUSATES ON THE SEPARATED FAST AND SLOW PIII RESPONSES OF THE ISOLATED FROG RETINA. MATSUURA, T. Dept. of Physiol., Kinki Univ. Sch. of Med., Sayama, Osaka 589

Separation of the fast and slow PIII from the aspartate-isolated PIII response was performed by the fractional recording and the effects of Ca^{2+}, TEA, papaverine and Ba^{2+} on both responses were investigated. Lowering the Ca^{2+} concentration in the perfusate up to 0.1 mM led to an increase in the peak amplitudes of the fast and slow PIII, whereas 10 mM TEA suppressed both responses. The application of 0.1 mM papaverine which is known to inhibit the cyclic nucleotide phosphodiestrase caused an increase in the amplitude of the fast PIII and a slowing down of the kinetics of the photoresponse. The treatment with 0.01 mM Ba^{2+} abolished completely the slow PIII with little effect on the fast PIII. The effect on the slow PIII was reversible within 20 min following return to the control perfusate. The light-induced $[K^+]_o$ decrease in the photoreceptor layer measured by the K^+-specific microelectrode was not affected during Ba^{2+} perfusion, implying that Ba^{2+} suppresses the slow PIII by its direct action on the K^+ conductance of the Müller cells.

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EFFECTS OF L-GLUTAMATE ON SOLITARY HORIZONTAL CELLS OF THE GOLDFISH RETINA.
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Effects of L-glutamate on membrane potentials of solitary horizontal cells, dissociated from papain-treated goldfish retinas and cultured in vitro, were studied either by bath-, pressure-, or electrophoretic application. L-glutamate produced a rapid and maintained depolarization which was dose-dependent (the least effective dose ca. 10 μM) and lacked any sign of desensitization. At higher doses, the glutamate-induced depolarization triggered action potentials which were similar to those evoked by electric stimulation. Responses to L-glutamate reversed in polarity at membrane potentials between 0 and -20 mV. The membrane resistance measured as a slope of the current-voltage relationships was lower in presence than in absence of L-glutamate. Responses to L-glutamate disappeared reversibly when extracellular sodium ions were replaced by choline ions. Neither L- nor D-aspartate produced any change in resting membrane potentials, while D- (but not L-) aspartate at relatively high doses (>1 mM) antagonized responses to L-glutamate. These results demonstrate that the chemosensitivity of horizontal cells to acidic amino acids persists after dissociation, and sodium ions contribute to the depolarizing response.

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RECEPTIVE FIELD STUDIES OF L-TYPE HORIZONTAL CELLS IN THE TURTLE RETINA.
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Luminosity (L-) type horizontal cell responses in the turtle (Geoclemys) retina were recorded intracellularly, and each cell was filled with horseradish peroxidase (HRP) by ionophoretic injection. Following histochemical reaction of the HRP, light microscopic inspection demonstrated that this type of cell has a cell body connected to a tuberous axon terminal by a fine axon. By examining the location of the cell in relation to the linear array of electrode penetrations, it was determined whether a given recording was made in the cell body or in the axon terminal. The size of the receptive field of the cell body was significantly smaller than that of the axon terminals. Although the distances between the cell body and the axon terminal of some L-type horizonal cells were as short as 50 μm, the length of the intervening axon was invariably longer and was at least 250 μm. Unless signals are amplified during their propagation along the axon, it is unlikely that current generated in the cell body spreads to the axon terminal through the thin axon, or in the opposite direction. The difference in receptive field sizes of cell body and of axon terminal may therefore indicate that these two structural subunits are isolated electrically.

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RESPONSES OF THE RETINAL HORIZONTAL CELL TO DENSITY-MODULATED RANDOM-DOT PATTERNS.
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Horizontal cell (HC) in the retina increases its response with flash intensity as described formally by a Michaelis-Menten (M-M) relation. Studied here in comparison with this are responses of the turtle L-type HC to flashing random patterns comprizing small dots of various calibrated densities. The peak responses against the mean flash intensity per unit area (proportional to the dot density) lay below the M-M curve, a finding attributable to the unequal inputs to individual photoreceptors coupled with another M-M aspect characterizing the photoreceptor transduction. Thus, microscopic contents of visual inputs within the receptive field also influence the spatial summation mechanism of HCs. However, if the HC responses of equal magnitudes are examined, they show virtually identical wave-forms no matter whether the stimuli were uniform or textured with dots. While this result was probably much due to mixing effects of the light scatter and the inter-receptor coupling, cones nonetheless showed varying amplitudes and wave-forms depending on their position relative to the random dots. This indicates that some averaging property of the HC also contributes to the wave-form invariance.

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A POSSIBLE NONLINEAR SPATIO-TEMPORAL MECHANISM FOR L-TYPE HORIZONTAL CELLS IN THE RETINA

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The response waveform to a brief flash of the L-type horizontal cell in the carp retina shows clear nonlinear time course, depending on the stimulus area or intensity. Also the response duration is reflected directly to the stimulus intensity, independent to the area stimulated. These basic nonlinear evidences have been simulated by the spatio-temporal discrete reduction mechanism (Usui et al., Vis. Res., 1983). We present here its physiological version. That is, introducing well known cell membrane physiology the discrete reduction mechanism can be interpreted through a two-dimensional network model with cell unit(Na-channel, K-channel and membrane capasitance) and nonlinear gap conductance. The model behavior shows clear characteristics not only its spatial properties but also its nonlinear dynamics described earlier. Depolarization in the recorery phase is responsible for the slow g_k dynamics. Improtant requirement to simulate the response nonlinear dynamics precisely is the hysteresis in the gap conductance. These assumptions should be tested physiologically and such experiments are now under studying.

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ON THE ROLE OF THE NEGATIVE FEEDBACK FROM L HORIZONTAL CELL TO RED CONES IN THE CARP RETINA.

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In 2mM Ba^{2+} containing solution, red components of R/G- and Y/RB-type horizontal cells were abolished. In some cases, the positive middle-wavelength components of Y/RB cell were also eliminated by Ba^{2+}. These results suggest that Ba^{2+} blocks the sign-inverting transmission in the pathways from L to R/G and from R/G to Y/RB cells. Blocking effect of this ion on the negative feedback from L to red cones was also investigated by comparing the L cell responses to red light modulated in a white-noise fashion, with and without application of Ba^{2+}. Ba^{2+} depolarized the membrane potential, enhanced the light responses and made a deviation of the response amplitude from the normal distribution. From these results and from an examination of the frequency responses of the light responses, it is suggested that the negative feedback from L cell to red cones plays a role in improving the frequency response as well as the linearity at the sacrifice of gain, like as the feedback in an electric circuit.

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ENTRAINMENT MECHANISMS IN THE CIRCADIAN LOCOMOTOR RHYTHM OF THE GIANT LAND SNAIL, ACHATINA FULICA.

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The giant land snail, Achatina fulica, shows the circadian freerunning and entrained rhythms in the locomotor behavior. In the present study, the activity patterns of intact snails were compared with those of eyeless animals under 24 hr cycles of light of three light intensities (400 lux, 100 lux and 3 lux). Intact snails exhibited the nocturnal entrained patterns under all of the light intensities. On the other hand, eyeless animals did not show the entrained patterns under the lower light intensities below 100 lux, although they were also nocturnally active at 100 lux. At 3 lux light, some intact snails exhibited a split freerunning rhythm. In this case, a 100-lux light pulse of 1 hr presented immediately before the dark-onset could entrain the locomotor rhythm.

These results suggest that the eyes play an important role both in generating and entraining the rhythm, especially under the lower light intensities below 100 lux.

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DIURNAL CHANGES IN ULTRASTRUCTURE AND ERG-RESPONSES OF THE COMPOUND EYE OF THE ISOPODE, *LIGIA EXOTICA*. HARIYAMA,T.and TSUKAHARA,Y.Reserch Center for Applied Information Sciences, Tohoku University, Katahira, Sendai 980

Spectral response curves based on ERG-measurements have two distinct peaks, one at around 380nm, the other at around 520nm. These two peaks seem to represent the activities of two classes of receptor cells identified by intracellular recordings. The size of the response to a green light of constant intensity depended on the time of a day, but that to UV-light was less affected. The ultrastructure of the retinula cells also changed diurnally and in response to light/dark adaptation. Irregular arrangements of microvilli appeared during the high phase in the ERG amplitude. Rhythmic pigment migrations were observed even in continuous darkness in most retinula cells except in two smaller ones (R4,R5) which possess the smallest amount of screening pigment granules. These results suggest that the green sensitive cells contribute dominaltly to the diurnal changes of the spectral response curve and an endogeneous mechanism is involved in manifestation of the morphological and physiological changes in these photoreceptors.

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MORPHOLOGICAL STUDIES ON THE TERMINALS OF THE VISUAL CELLS OF JAPANESE DACE. UEKI, K. and HASHIMOTO, Y. Dept. of Physiol. Tokyo Women's Med. College, Shinjuku-ku, Tokyo 162

There are two types of the terminals of the visual cells of Japanese dace. One is small in size and has only one ribbon synapse in it, and the other is large in size and contains many synaptic ribbons. From the electronmicroscopic study on the HRP-injected C-type horizontall cells, the processes of RB-type horizontal cell contact not only with the large terminals but also with many small terminals. The processes of RGB-type horizontal cell, on the other hand, contact with only the small terminals. As previously reported, unequal double cones and two kinds of single cones show a very regular row mosaic patterns. All their terminals seemed to be large. Other members of the cone cells are socalled oblique and miniature cones, (under microspectrophotometric work we called them "short single cone"), and they seemed to have small terminals under the investigation of the isolated single cone, though it needs further study. Rod terminals are small in size as usual.

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CONE ABSORPTION AND HORIZONTAL CELL RESPONSES IN THE JAPANESE DACE. Hashimoto, Y., Koike, K., Harosi, F. I.*and Collins, B.-A.*Dept. of Physiology, Tokyo Women's Medical College, Shinjuku-ku, Tokyo 162, and *Marine Biological Laboratory, Woods Hole, MA. 02543, USA.

The C-type horizontal cells in the Japanese dace retina showed the characteristic spectral responses with large hyperpolarization to short wavelengths. It suggest that this retina contain very short wavelength-absorbing pigments. We investigated the photoreceptors using a dichroic microspectrophotometer. We found four types of cone and one type of rod pigment. Long member of double (LD) and the large single (LS) cones contained a red-absorbing pigment peaking in the range of 570-620 nm. The short member of double (SD) and medium size single (MS) were green-absorbing, peaking in the range of 500-535 nm. The small single (SS) were of two types. The slightly larger (SS-1) cones had a violet-absorbing pigment with λ max of 410-420 nm. The smallest cones (SS-2) were ultraviolet-absorbing with λ max of 360-370 nm. The rods were of a relatively long-wavelength type with λ max of 515-535 nm. There were strong correlation between pigment content and cone morphology. The largest cones were absorbing the longest wavelengths, whereas the smallest and most tapered outer segments had the UV-absorbing pigment. We could not find the blue-absorbing pigment. A simulation for the horizontal cell spectral responses using these visual pigments will be described.

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THE INFLUENCE OF FMN ON THE REGENERATION OF RHODOPSIN IN RETINA . FUJISHITA, S.
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FMN and rhodopsin were extracted from the left and right eyes of a frog respectively. FMN and rhodopsin per 1g of retina are expressed as χ x 10^{-5}mol/g and y (optical density) respectively. From the data of χ and y obtained from 20 frogs, the relation between χ and y may be expressed as follows : $y = 2.912 + 1.392$. And the correlation coefficient between χ and y is calculated as 0.398. This value indicates that increase of FMN in a retina causes the regeneration of rhodopsin in the retina. The reason why the increase of FMN in a retina causes the increase of rhodopsin may be explained as follows : 11-cis retinal synthesized in the retina, combinedwith opsin spontaneously. So, when the conversion from 11-cis retinol 11-cis retinol is facilitated by the activity of alcohol dehydrogenase, regeneration of rhodopsin may be increased. On the other hand , the activity of the enxyme may be accelerated by the action of FMN of a certain flavin enzyme. So, the amount of rhodopsin in retina may be influenced with the amount of FMN in the retina.

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FUNCTIONAL ROLE OF CYCLIC-GMP IN PHOTOTRANSDUCTION MECHANISM IN GECKO PHOTORECEPTORS. KAWAMURA. S. AND MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo.

Cyclic-GMP was iontophoretically injected into the gecko photoreceptor from one barrel of a double-barrelled microelectrode, while the other barrel was monitoring membrane potential and conductance changes. Cyclic-GMP induced a prolonged depolarization accompanied by a marked increase of membrane conductance. The duration of the depolarization was dose-dependent. The time course of repolarization was gradual in the dark, but was accelerated when a light flash was given, probably because injected cyclic-GMP was rapidly reduced in the light. The cells, which had been tested to be sensitive to cyclic-GMP, produced no significant potential changes with injection of acetate or 5'-GMP (a hydrolytic product of cyclic-GMP), indicating that the depolarization was cyclic-GMP-specific. The depolarization caused by cyclic-AMP injection was so small that cyclic-AMP seemed unlikely to be an internal transmitter of photoreceptors. In the sodium-free bathing solution (substituted by choline), the depolarization never occurred with injection of cyclic-GMP, suggesting that cyclic-GMP is involved in the gating mechanism of sodium channels in the photoreceptor membrane. These results support the cyclic-GMP hypothesis currently proposed for phototransduction mechanisms.

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ANALYSIS OF ROD SYSTEM IN FROG RETINA BY MEANS OF TRANSRETINAL CURRENT APPLICATION. MIYACHI, E.-I., TAKAHASHI, K.-I. AND MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo.

In carp cones, a GABA-mediated IPSP was detected when horizontal cells were depolarized by application of a transretinal current pulse, indicating that a negative feedback operates from horizontal cells to cones in the dark, and ceases its function in the light (Murakami et al., 1982). In order to examine whether a negative feedback is present or absent in the rod system, similar experiments were carried out on the retina of the bullfrog, Rana catesbeiana. Responding to a transretinal current pulse flowing from the receptor side to the vitreal side, the rod produced a biphasic deflection; a transient depolarization followed by a hyperpolarization. Both phases were simultaneously diminished when the rod membrane was depolarized, suggesting that both have the same equilibrium potential in common. Following experiments in various ionic media indicated that the transient depolarization seemed identical to a Ca-spike accompanied by an after-hyperpolarization, which has been reported in photoreceptors of other animal species. Many attempts, however, failed to adduce evidence in support of the presence of a negative feedback in the rod system of the frog.

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MEAN LIFE TIME OF THE OPEN CHANNEL IN THE PHOTORECEPTOR MEMBRANE OF THE MANTIS SHRIMP, SQUILLA ORATORIA.

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Photoreceptor cells of the mantis shrimp, Squilla oratoria, were studied under voltage clamp at 6°C-22°C. Current fluctuations induced by light were analysed. Power spectrum of current noise found to fit a simple Lorentzian curve. As the stimulus light intensity (I) increases, the value of the zero-frequency asymptote of the spectrum becomes larger in proportion to I when the light is dim and becomes smaller at the bright light which elicits the response larger than $V_{max}/2$. The elementary conductance and mean open time (τ) of the channel are 8.2 pS and 14.4 msec at 19°C, respectively. The membrane potential from -65 mV to -5 mV does not affect the τ value, which strongly depends on I. Intracellulary injected Na^+,K^+,Cl^-, and 5'GMP do not change the τ value within the error. The small but significant effect on τ is observed after cAMP injection. EGTA and cGMP double the τ value. The effect of EGTA is irreversible, while that of cGMP disappears in 5 to 7 minutes. A phosphodiesterase inhibitor (IBMX) applied to the superfusate also prolongs τ. Arrenius plot of the corner frequency against the reciprocal of the absolute temperature is linear and the slope of the line corresponds to an Arrenius activation energy of 7.02 Kcal/mol. These results suggest that Ca^{++} and cGMP play important roles to control the life time of the open channel.

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EFFECTS OF COBALT IONS ON PHOTORESPONSES IN FROG RODS.

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Rod membrane potential oscillations (ca. 2 Hz) following to light-elicited response have been shown as inherent activities in the light-adapted retinas of bullfrog (Rana catesbiana). Membrane currents constituting the oscillations were studied by intracellular recordings of membrane potentials and the application of cobalt ions. When light-adapted retinas were perfused with Co^{2+} (0.1 mM) containing Ringer, recovery of flash response to the pre-stimulus level was markedly delayed in comparison with that of control response in normal Ringer. This delayed recovery was always accompanied by the disappearance of oscillatory activity following to initial light-evoked hyperpolarization. On the other hand, the rate of rise of photoresponses, shape of response amplitude-light intensity curves, and relative sensitivity to the light, however, were not affected at all by cobalt ions. These results suggested that faster response recovery observed in light-adapted rod might be correlated with the change of Co^{2+}-antagonized calcium current at least in part.

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CHANGES IN THE DARK CURRENT ACCOMPANYING THE OFF-RESPONSE OF VISUAL CELLS.

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A prolonged weak light illumination has been reported to induce an off-response in transretinal potential of bullfrog retina immersed in a low Ca^{++}, Ba^{++} and Na-aspartate containing solution. To test the assumption that the off-response is due to a transient activation of Na-pumps located in rod inner segments, light induced changes in current flowing along photoreceptors were studied by inserting a pair of micropipettes into the bullfrog retina.

Changes in the dark current induced by a stimulus light of 30 sec consisted of the on- and off-component. The amplitude ratio of off- to on-components was about one when tips of electrodes were positioned near the rod outer segments, and increased as they were advanced to the inner segment layer. In the absence of Ba^{++}, similar changes in currents were also observed, though no detectable off-response was observed in the transretinal potential. Findings suggest that regardless of the presence of Ba^{++}, current flowing outward through the plasma membrane of the rod inner segments increases transiently when the off-response is developing.

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EFFECT OF TEMPERATURE UPON THE REFRACTORINESS OF A PHOTORECEPTOR POTENTIAL RECORDED FROM THE HORSESHOE CRAB LATERAL EYE. KIKUCHI, R. Dept. of Physiol., Tokyo Women's Med. Coll., Shinjuku-ku, Tokyo.

The amplitude of the response to the test flash decreased as the interval between the test and conditioning flashes of same intensity and duration after a few minutes in darkness. If the double flash interval was fixed at a given period, the relative sensitivity of the photoreceptor to the test flash decreased to a certain extent as the flash intensity increased. The depressed sensitivity was increased under hyperpolarizing currents during test flashes. The shorter the flash interval was, the greater the current effect was. The current effect was greater at lower temperatures. The amplitude of the steady depolarization by prolonged illumination was decreased by hyperpolarizing currents during the illumination to a certain extent. The decrease in the relative sensitivity was partially recovered in a Cs-Ringer. The time course of the recovery of relative sensitivity was prolonged by lowering temperature and the amplitude of conditioning and test flash responses decreased with a rather high temperature coefficient. Factors responsible for these changes were discussed.

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DEPENDENCY OF RESPONSE SHAPE OF RODS UPON WAVE LENGTH OF STIMULATING LIGHT. TANAKA, I., TAUCHI, M.* and NAKATANI, K.** Research Institute, Natl. Rehab. Center for the Disabled, Tokorozawa, Saitama, *Dept. of Information Physiol., Natl. Inst. Physiol. Sci., Okazaki, Aichi, **Dept. of Physiol., tokyo Women s Med. Coll., Shinjuku-ku, Tokyo

The intracellular responses of red rods in the retina of the bullfrog, Rana catesbiana, were studied with brief flashes of monochromatic light (from 400 to 655 nm) in steps of 51 nm covering a circle of 3 mm in diameter. The log intensity-response amplitude relation for the each light depended upon wave length; i.e., a simple shift of the log intensity-response amplitude curves for 400 and 655 nm light along the intensity axis could not be fit perfectly on that for 502 nm. Spectral sensitivity of rods depended upon the criterion voltage. The sensitivity to red light was much greater than that predicted by the absorption spectra of rhodopsin and porphyropsin. The shapes of response to 400 and 655 nm light were markedly different from that to 502 nm light under the same response amplitude. It is concluded that the rod response does not seem to fit "the principle of univariance" in respect with chromaticity and that red rods receive excitation from neighboring cones or cones and green rods.

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EFFECTS OF INTRACELLULAR INJECTION OF EGTA AND TEA ON INSECT PHOTORECEPTOR POTENTIAL.
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EGTA or TEA was injected iontophoretically into the retinula cell of locust.

(1) Both increased the size of the sustained phase of the light coincident receptor potential.
(2) After TEA injection, an intense stimulating light induced a depolarizing afterpotential.
(3) On the contrary, EGTA did not develop the depolarizing afterpotential, rather it enhanced a slow hyperpolarizing afterpotential.
(4) Moreover, the EGTA injection suppressed the depolarizing afterpotential which was induced after TEA injection.
(5) It is suggested that the depolarizing afterpotential results from an increase in the concentration of intracellular Ca ion and the enhancement of the afterhyperpolarization is caused by a decrease of it.

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PARTIAL DIFFERENCE OF PHOTOTRANSDUCTION PROCESS IN INSECT PHOTORECEPTOR.

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This study was performed to obtain a cue for solving a problem of two high sensitivity peaks at ultra-violet and blue-green in spectral sensitivity curve of the fly predominant photoreceptor (R1-6). Large or sharp-form bumps were elicited by ultra-violet light when electrodes were inserted into proximal part of the receptors and were elicited by blue-green light when electrodes were inserted into distal part. Latency of bumps and late receptor potentials evoked by weak ultra-violet light was shorter in proximal part than in dorsal part, while that evoked by weak blue-green light was shorter in dorsal part than in proximal part. Two-dimensional polyacrylamide gel electrophoresis identified several light-induced proteins. Among them a few classes of polypeptides showed different wavelength-dependent changes in proximal and distal parts of the photoreceptor, corresponding to the results obtained electrophysiologically. These results demonstrate a partial difference of phototransduction process within one receptor cell.

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EFFECTS OF ACOUSTIC MIDDLE EAR REFLEX ON SOUND TRANSMISSION AND CHARACTERISTICS OF ITS REFLEX ARCH IN RATS. MURATA, K., ITO, S. and HORIKAWA, J. Dept. of Neurophysiology, Medical Research Institute, Tokyo Medical and Dental Univ. Chiyoda-ku, Tokyo 101

Change in transmission through the middle ear of albino rats anaesthetized with chloralose was assessed from change in amplitude and phase of cochlear microphonics (CM). In an ear the assessment was made before and after inactivating the stapedius (ST) or tensor tympani(TT) muscle, while the other ear was acoustically stimulated to elicit the reflex. Transmission loss due to ST muscle contraction was constant and largest below 1kHz. The loss decreased with frequency increase up to 10-20kHz, beyond which there was observed slight loss or not any more. The frequency characteristics of loss and phase-shift obtained could be modeled with a high-pass filter consisting of a variable capacitor and resistor in parallel with an addition of another resistor in series. The TT muscle was far less effective than the ST muscle, and the former caused only slight loss at all frequencies except between 10 and 20kHz, in which loss amounted 5dB or less. The transmission characteristics of open-loop reflex arch was measured from change in amplitude and phase of CM envelope in response to reflex-eliciting tones to the opposite ear, the tones being amplitude-modulated with a sinusoid at various frequencies. It seemed to be approximately a 2nd or higher order low-pass filter with a cut-off frequency of 20-30Hz.

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DECREMENTAL RESPONSE AT HAIR CELL-AFFERENT FIBER SYNAPSE IN GOLDFISH'S EAR. FURUKAWA, T. and SUZUE, T. Dept. of Physiology, Tokyo Med. & Dent. Univ. Sch. of Med., Yushima, Bunkyo-ku, Tokyo 113.

Decremental response is a transient reduction in the amplitude of the EPSPs (or in the rate of afferent firings) brought about by a small decrement in the sound intensity. It can be attributed either to a transient hyperpolarization (which is not so likely either) or to processes at the afferent synapse. Present study pertains to the latter alternative. We tried to see with computer simulation how far the decremental response could be explained from our scheme proposed for the mode of operation of hair cell synapses, regarding the release, depletion and replenishment of transmitter quanta (Furukawa et al.: J. Physiol. , 322, 181, 1982). Simulation was made with a microcomputer using a program written with the BASIC. After repeated trials and revisions, we could demonstrate that input-output relations including adaptation and incremental response could be simulated fairly well. In comparison to that, however, reproduction of the decremental response remained less satisfactory.

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SOUND EVOKED FIRINGS OF GOLDFISH AUDITORY FIBERS AS RELATED TO AMPLITUDE OF EPSPS KUNO, MIYUKI Dept. of Physiol., Osaka City Univ. Med. Sch., Abeno-ku, Osaka 545

In various animals, sound evoked impulses of auditory fibers show some characteristic firing patterns, such as, adaptation, incremental and decremental responses. In this study, to demonstrate the factors participating in the formation of firing patterns, simultaneous recordings of spike potentials and excitatory postsynaptic potentials(EPSPs) were conducted extracellularly with glass microelectrodes from S1 fibers of goldfish auditory nerve. Curves of firing probability plotted against the order of stimulus sound waves were almost parallel for afferent impulses to different intensities and frequencies of sound. Low frequency and high intensity sound sometimes induced "saturation firings" for a rather long period. Amplitude of suprathreshold EPSPs was estimated from the notch on the rising phase of spike potentials or changes in spike height. The mean amplitude of EPSPs was linearly related to the firing probability over the limited range (firing probability: between 0 and 100%) in both adaptation and incremental responses of afferent firings. On the other hand, the threshold for spike initiation of the fibers was unchanged. The results demonstrated that changes in firing probability of the auditory fibers are largely attributed to those in the mean amplitude of EPSPs and not to changes in excitability of the fibers under various conditions of sound stimulation.

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EFFECTS OF STATIC HAIR BENDING OF AUDITORY HAIR CELLS ON THE FIRING RATE OF SPONTANEOUS AND SOUND-EVOKED IMPULSES OF GOLDFISH AUDITORY FIBERS.
Kyogoku, I. Kuno Miyuki & Matsuura, S. Department of Physiology, Osaka City University, Medical School, Abeno-ku, Osaka 545

Auditory afferent fibers of various animals including mammals and Ostariophysi show spontaneous discharges. In this study, static bendings of sensory hairs were applied to the hair cells to investigate the changes of firing rate of spontaneous and sound-evoked impulses of S2 fibers of goldfish during the hair bending. When a sustained hair bending was applied by giving a continuous pressure to the swim-bladder indirectly from the surface of fish body, sound-evoked afferent impulses decreased in firing rate of about half of the fibers tested, and increased in other half of fibers. Spontaneous discharges also decreased when hair bending was applied toward a direction which suppressed firings of afferent impulses to sound. Hair bending toward another direction increased both firing rates of spontaneous discharges and afferent impulses to sound. The observations suggest that synaptic drive from hair cells to S2 fibers, due to static activities of hair cells, may be involved in the generation of spontaneous discharges.

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THE RESPONSE OF THE AMPHIBIAN PAPILLA NERVE OF THE TOAD IMMOBILIZED WITH D-TUBOCURARINE.
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Single unit responses to tone stimuli in the amphibian papilla nerve fibers were compared between immobilized animals(IA's:25mg/kg d-tubocurarine chloride(d-TC) applied intramuscularly) and anesthetized animals (AA's:2.5g/kg urethane injected into the dorsal lymph sac). Most units obtained in the IA's were spontaneously firing, while most units obtained in the AA's were silent when tone stimulus was absent. The distributions of characteristic frequencies, and those of values of Q_{10dB} were similar in both preparations. The mean of the maximum firing rates(MFR's), however, was significantly higher in the IA's than in the AA's(156.7 ± 48.6 vs. 76.9 ± 40.3 imp/sec). When d-TC was applied intramuscularly or locally into the perilymphatic space in the AA's,5 units out of 16 showed significantly high MFR's as units in the AA's. In the IA's, the MFR generally decreased by an injection into the lymph sac, but did not by the local application, of urethane.

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FIRING RATE OF SINGLE UNIT DISCHARGE OF THE AUDITORY NERVE FIBER DURING PERFUSION OF THE BULLFROG PERILYMPHATIC SPACE.
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Single unit activities were recorded from the auditory nerve fibers innervating amphibian or basilar papilla (AP or BP) in the bullfrog. The perilymphatic space was perfused with frog Ringer's solution, 2 minutes with and 2 minutes without sound stimulation. Then the source and sink of the perfusion was exchanged, that is, the direction of the pump rotation was reversed. Thus, effectively, perfusate collected during the perceding period of 4 minutes was reperfused for the next period. In the reperfusion of 52 units, the spontaneous discharges increased in 12, dicreased in 14, and did not change in 26 units, significantly. The data of AP and BP fibers are mixed, as it seemed that no specific difference was present between the two groups. Results could not yet confirm the report of Sewell et al. (1978), which suggested release of some auditory nerve-activating substance in the perilymph of animals stimulated by sound.

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TONOTOPIC ORGANIZATION IN BULLFROG AMPHIBIAN PAPILLAE STUDIED BY HORSERADISH PEROXIDASE INJECTED WITHIN THE EIGHTH NERVE GANGLION. YANO, J., OOYAMA, H., SUGAI, T. and CHUJO, T. Dept. of Physiol., Kanazawa Med. Univ., Uchinada, Ishikawa

Horseradish peroxidase was applied to the primary auditory neurons in the bullfrog eighth nerve ganglion to label single nerve fibers after identification of innervation to the amphibian papilla(AP) and determination of characteristic frequency(CF). Diaminobenzidine(DAB) and tetrametylbenzidine(TMB) were used as chromogen in histological procedures. Two fibers with low CF(50 and 140 Hz), labeled with black DAB reaction product, were found near the anterior macula. CF's of six fibers detected by a granular appearance of the characteristic TMB reaction product were in the range from 150 to 700 Hz. These fibers with low and high CF's were found near the anterior and posterior portions of the macula, respectively. Thus, in the AP of the bullfrog, primary auditory neurons with low CF may innervate the anterior macula and as increase of the CF, innervation sites may move caudally along the longitudinal macular axis.

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TEMPORAL INTERACTION BETWEEN EXCITATORY AND INHIBITORY ACTIVITY IN RESPONSE TO REPETITIVE TONE BURSTS IN INFERIOR COLLICLUS NEURONS OF CATS. ITO, S. and MURATA, K. Dept. of Neurophisiol., Med. Res. Inst., Tokyo Med. and Dent. Univ. Chiyoda-ku, Tokyo

Responses to trains of short tone bursts (BT) at various repetition rates of the best frequency were recorded with a tungsten electrode. Probability of firing of fast adapting on-neurons in response to successive bursts subsequently decreased more abruptly with increase in repetition rate of bursts and they responded to a BT of supramaximal rate like to a long burst (LB) of the best frequency. In contrast, fast adapting off-neurons showed successive increase in the firing probability at each succeeding bursts of a moderate rate and fired only at the end of BT at higher rates. These facts might imply that the on-response was followed by a short inhibitory process whose effect summated during a BT stimulation and that a excitatory process which caused the off-response lasted more or less to summate in a BT stimulation. Neurons responding to a LB with an initial silent period of 10msec or less and with succeeding sustained firing were activated synchronously with each burst of a low rate BT. They were inhibited nearly completely by a BT of an appropriate rate, though a higher rate BT elicited the same response as to LB. This may be explained by competition between dominant but slowly summating excitatory process and rapidly summating inhibitory process during repetitive stimulation.

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NEURONAL PROCESSING OF TEMPORAL PARAMETERS OF SPEECH SOUNDS IN THE MEDIAL GENICULATE BODY. HASHIMOTO, T. Div. Biocybernetics, Inst. Med. Dent. Engin., Tokyo Med. Dent. Univ., Chiyoda-ku, Tokyo 101.

The neural representation of speech parameters was investigated in the medial geniculate body of unanesthetized guinea pigs. Single unit responses were recorded to a speech-like sound, which was synthesized by a temporal combination of a formant analog, a random noise, a single click and a sinusoidal click train or tone burst with frequency sweep. They correspond to a vowel, a fricative, a plosive and a time-varying feature of articulation, respectively. The decoding of the temporal feature of speech sounds may be essential for the neural recognition of thw natural continuous speech. One medial geniculate neuron was selectively tuned to the time interval between the preceding consonant and the following vowel. The neuron may be specified as a C-V detector for the continuous speech. Another geniculate neuron was responsive sensitively to any change of features in the stimulus speech-like sound. The neuron may be specified as a segment detector for the continuous speech recognition. It may play an important role in the the neural mechanism of speech recognition with the combination of phoneme detectors.

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FREQUENCY AND AMPLITUDE REPRESENTATIONS IN THE ANTERIOR REGION OF THE PRIMARY AUDITORY CORTEX OF THE MUSTACHED BAT. ASANUMA,A. and SUGA,N.* Department of physiology, Dental School, Thurumi University, Tsurumi-ku, Yokohama. *Department of Biology, Washington University, St.Louis, MO, U.S.A.

Tonotopic and amplitopic representations were examined in the anterior primary auditory cortex of the mastached bat. Extracellular recording of neuronal responses from the unanesthetized animal was obtained from free-field stimulation with pure tones, FM sounds, and signals simulating their orientation sounds and echoes. Neurons responded strongly to single pure tones at short latency, but showed no facilitative response to paired stimuli. Neurons with best frequencies from 110 to 90 kHz were tonotopically organized rostrocaudally, with a disproportionate representation at 91-95 kHz. A striking discontinuity in the frequency representation from 90 to 64 kHz was found across the anterior-DSCF border. Most neurons exhibited monotonic impluse-count functions and responded maximally to sound greater than 80 dB SPL. There were also neurons that responded best to weak sounds, but amplitopic representation was not found.

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CORRELATION BETWEEN VOCALIZATION AND AUDITION IN THE JAPANESE HORSESHOE BAT. TANIGUCHI, I. Medical Research Institute, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113

An orientation sound (pulse) of the Japanese horseshoe bat (Rhinolophus ferrumequinum nippon) consists of a constant frequency (CF) component followed by a short FM component. CF was 65.5 kHz on an average of 6 animals (65.2 - 65.8 kHz). On the other hand, the best frequency (BF) at the lowest threshold in audiograms as measured by the pinna reflex was 66.3 kHz (65.8 - 67.3 kHz). The frequency difference (Δf) between CF and BF was 0.8 kHz (0.2 - 1.2 kHz). Audiograms showed sharp cut-off on both sides near BF. When a bat and a target are at rest, the frequency of echo is the same as that of an emitted pulse. Therefore, the bat must compensate Δf for echolocation. When the bat hanging from a toe-hold emits numerous pulses, the bat moves the pinnas reciprocally towards the target. The velocity of the pinna movement seemed to be high enough to compensate Δf by Doppler shift. The pinna in the CF-FM bat, Rhinolophus, can function not only to localize but also actively to modulate returning echoes of its own pulses.

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FACILITATION AND INHIBITION OF AUDITORY EVOKED POTENTIALS BY A CONTINUOUS PURE TONE. NOMOTO, M. Dept. of Physiol., Med. Sch., Dokkyo Univ., Mibu, Tochigi-Ken 321-02

In the Field L of the pigeon, auditory evoked potentials (AEPs) in response to clicks were increased, decreased, both increased and decreased, or unaffected by stimulation of background continuous pure tones depending on specific frequencies. In the nucleus angularis (NA) and nucleus magnocellularis (NM), AEPs in response to clicks were only suppressed at all of the recording positions by a continuous pure tone. In the nucleus mesencephalicus lateralis pars dorsalis (MLD), the amplitude of AEPs to clicks was mainly decreased and both decreased and increased by application of continuous pure tones. Single and multiple unit activity also compared with the AEPs in the MLD. Although the unit activities did not necessarily consist with the behaviors of AEPs, a pure tone that produced inhibition in a given unit caused facilitation of the impulses in response to clicks, whereas a pure tone which exhibited excitation brought about inhibition of the impulses to clicks. The relations between frequencies of continuous pure tones that produced the largest suppression and best frequencies that elicited the maximum response to pure tone bursts in the NA and NM were different from those in the MLD and Field L. The data suggest that facilitation and inhibition of the AEPs in the Field L are due to the neural mechanisms of the upper auditory system.

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ACOUSTIC RESPONSE CHARACTERISTICS OF NEURONS IN THE PRIMARY AUDITORY CORTEX (AI) OF THE CAT : AN INTRACELLULAR POTENCIAL STUDY MITANI,A. & SHIMOKOUCHI,M. Dept. of Behavioral Physiology, Faculty of Human Sciences, Osaka Univ., Suita, Osaka 565

Acoustic properties of AI neurons were studied intracellularly in immobilized cats using sealed stimulating system incorporating microphone assemblies.

A total of 65 neurons were recorded; 62 neurons responded to pure tones, and 3 neurons were completely uninfluenced. The majority of neurons (53 neurons) responded with on- and offset EPSPs. Five and 4 neurons responded with sustained EPSPs and onset IPSPs - offset EPSPs respectively, and approximately 50% of the 62 neurons showed spike potentials. The minimum onset latencies were about 8 msec. Most of 62 neurons responded to pure tones had broad and irregular tuning curves, and a small proportion of neurons exhibited sharp tuning curves. Some recorded neurons were visualized by intracellular HRP injection method. Pyramidal cells in layer II had no response to pure tones and pyramidal cells in layer III showed response patterns with irregular tuning curves.

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FUNCTION OF THE BAND OF NOISE UNITS IN THE AUDITORY CORTEX IN CATS. MARUYAMA, N., SAITOH, K. and KUDOH, M. Dept. Neurophysiol., Brain Research Inst., Niigata Univ., Niigata 951

About 50% of the auditory cortex units were band of noise (BN) units which exhibited sustained responses to BN in cats. Based upon the best bandwidth, these units were divided into 3 types; wide (W), medium (M) and narrow (N) type. Effective ranges of the center frequency of BN extended over a very wide frequency range in most cases. In the present experiments, we found a few BN units where the effective range of the center frequency was restricted. Spectrums of some cat's cries such as hissing and purring are similar to those of human fricative consonants in which sound energy is distributed continuously within a restricted frequency range. These BN units might act as detectors of fricative consonants. We also investigated the responses of BN units to saw-tooth AM tones. The best modulation frequencies were about 200 Hz or more for W type units, 30 - 60 Hz for M type units and about 10 Hz for N type units. Some of M type units had more than one peak for modulation frequencies in terms of driven discharge.

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VOWEL SENSITIVE NEURONS AND ITS VOWEL DETECTING MECHANISM. MARUYAMA, N., SAITOH, K. and KUDOH, M. Dept. Neurophysiol., Brain Research Inst., Niigata Univ., Niigata 951

We have already reported on the cat auditory cortex neurons which are thought to be involved in detecting vowels. They are the vowel sensitive neuron and the vowel detector. The former is much more responsive to specific combinations of 2 synthesized formants (SFs) than to single SFs. The latter is inhibited by single SFs but respond exclusively to specific combinations of 2 SFs. In the present experiment, we studied on the response of a vowel sensitive neuron to various combinations of 2 SFs and made an iso-spike-count contour map on the 1st formant frequency - 2nd formant frequency ($F_1 - F_2$) plane. Although the range of combinations of 2 SFs surveyed was not sufficiently extended, the most dominant peak was not at the combination of 2 single SFs at the respective peaks.

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DEPENDENCE OF CM REDUCTION OF TEST TONE LEVEL IN ACOUSTIC FATIGUE.

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Study was made to reveal the process lying under the decrease with the test tone level (TTL) of CM reduction in acoustic fatigue, which phenomenon was first reported by Lerche-Münster (1955) and remained unsolved of its origin. Stimulus tone frequency was common to fatiguing and test, mainly 5 kHz. CM was recorded from the first turn of the guinea-pig cochlea. The fatigue input-output function was characterized by linear rise below the departure from linearity (M_L) and by sigmoid rise above M_L, which attained to the control function curve near the maximum if the reduction in the linear range was not large, 50% for instance. The attainments of several functions of different reduction values converged almost completely. The change in the form of fatigue recovery curve with TTL from lower to higher under a constant fatiguing load corresponded with that of fatiguing load change from strong to weak under a low constant TTL. All the findings above cannot be explained as an artefact or non-mechanical processes (the sigmoid rise occurs at the SPLs which cause fatigue). Thus we are forced to conceive the occurrence of some mechanical process by which the test tone less receives the effect of fatigue with the increase of TTL, and only possible event would be the basalward shift of the peak of the vibratory pattern of the cochlear partition. Furthermore the convergence strongly suggests a sharp peak of the pattern in the linear action.

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EFFECTS OF SYMPATHETIC STIMULATION ON PERIODONTAL MECHANORECEPTOR IN THE CAT. KARITA, K., TABATA, T. and AOKI, T. Department of Physiology, Tohoku University School of Dentistry, Seiryo-machi, Sendai 980

EMG from digastric muscle evoked by mechanical stimulation of the canine tooth was found to be suppressed by electrical stimulation of cervical sympathetic trunk. In order to analyze the receptor mechanism of this inhibitory effect following experiments were made. The activity from periodontal mechanoreceptors was recorded in a single fiber or whole of infraorbital nerve innervating a maxillary canine tooth in the anesthetized (Nembutal,50mg/Kg) cat. The discharges of the mechanoreceptors to a controlled force applied to the tooth were suppressed by methoxamine (α-adrenergic agonist) applied to the apical area of tooth after the pulpectomy. Electrical stimulation at distal end of cut infraorbital nerve also inhibited the response to mechanical stimulation of the tooth. On the contrary, spontaneous activity propagated from mechanoreceptors was enhanced by methoxamine. These results suggest that activity of periodontal mechanoreceptors is inhibited by sympathetic supply and that the spontaneous activity is not necessarily due to the elimination of sympathetic inhibitory effect.

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AUGMENTATION OF CHEMICAL RESPONSES OF THE POLYMODAL RECEPTORS BY PG-E_2AND 5-HT. MIZUMURA, K. and KUMAZAWA, T. Dept. Nervous and Sensory Functions, Research Institute of Environmental Medicine, Nagoya Univ.,Chikusa-ku, Nagoya 464, Dept. Physiol., Nagoya Univ. Sch. Med., Showa-ku, Nagoya 466

Effects of prostaglandin-E (PG-E_2) and 5-hydroxytryptamine(5-HT), presumed potentiators in inflammed tissues, on activities of polymodal receptors were studied using in vitro testis-superior spermatic nerve preparations excised from anesthetized dogs. PG-E itself never excited polymodal receptors at a concentration of 0.05 μg/ml, but above 0.5 μg/ml it had a weak but concentration dependent excitatory action on some of polymodal receptors. PG-E_2(0.05 μg/ml in most cases) augmented responses of polymodal receptors to bradykinin(0.1 μg/ml), 60 mM KCl solution and 3.6% NaCl solution, but a longer application time or higher concentration was needed for the latter two substances. 5-HT (0.2-25 μg/ml) as well enhanced responses to algesic substances, though 5-HT alone seldom evoked discharges of the receptor up to a concentration of 25 μg/ml. Activation effect of 5-HT, if any, was not concentration dependent. Since reported concentrations of PG-E_2in some of inflammed tissues and those of 5-HT in the whole blood are close to those used in this experiment, it is quite probable that the sensitivity of polymodal receptors is enhanced by PG-E_2 and/or 5-HT in inflammed tissues.

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SUPPRESSIVE EFFECTS OF SUBCUTANEOUS THERMAL STIMULATION AND INTRAMUSCULAR INJECTION OF BRADYKININ ON THE JAW OPENING REFLEX IN THE RAT. KAWAKITA,K. and FUNAKOSHI,M. Dept. of Oral Physiology, Gifu College of Dentistry, Hozumi-cho, Motosu-gun, Gifu 501-02

The effects of subcutaneous heat stimulation and intra-muscular injection of bradykinin(BK) on the jaw opening reflex(JOR) were examined. The metal piece for heating was placed on the lateral side of lower leg muscles. The JOR was clearly suppressed by the transient subcutaneous heating (60s) and the degree of suppression was increased with the increase of heating temperatures from 40 to 47.5°C with some after-effects. The repetitive application of heating of 40°C at intervals of 10 min tended to enhance its effect and that of 45°C tended to diminish its effect. The premedication of naloxone(10mg/kg) partly antagonised the heat-induced JOR suppression. Heating of the skin was almost ineffective below 45°C, and a weak suppression was induced by over 47.5°C of heating. The intra-muscular injection of BK(20ug) had similar effects on the JOR. These results suggest that the receptors which responsive to subcutaneous heating and BK injection participate in the process of JOR suppression and it is partly mediated by endogenous opiate systems.

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EFFECTS OF ACUPUNCTURE STIMULATION ON THE ELECTRICAL ACTIVITY OF RAPHE-SPINAL NEURONS IN RATS. KAZUO TODA and MICHIKO HAMBA , Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., and Dept. Physiol., Sch. Dent., Showa Univ ., Tokyo.

Extracellular recording was made from single cells in the nucleus raphe magnus (NRM) which project to the spinal cord. The spontaneous firing rate of these cells increased (63% of the observed cells), decreased (20%) or was not altered (17%) after acupuncture stimulation. The majority of the cells (78%) responded with spikes to stimulation of periaqueductal gray matter (PAG), indicating excitatory projections from PAG to NRM. When naloxone was iontophoretically applied to the NRM cells, antagonizing effect was seen in the acupuncture-inhibited cells but was not in the excited ones. Iontophoretically applied morphine depressed the activity of acupuncture-inhibited cells. The firing index of the antidromic excitation of raphe-spinal neuron was not changed by iontophoretically administered naloxone or morphine, suggesting that endogenous opioids released by acupuncture do not have the direct action on the NRM cells. By contrast, naloxone-microinjection into the PAG antagonized the acupuncture-induced changes in both the acupuncture-excited and the inhibited NRM cells. We conclude that (i) excitatory responses of the NRM cells are produced by activation of PAG after acupuncture and the synaptic transmission between PAG and NRM is presumably non-opioids (ii) the inhibitory responses are also produced by PAG activation and this inhibition is modulated by presynaptic opioids action.

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ACUPUNCTURE EFFECTS ON THE NEURONAL ACTIVITY OF THE ARCUATE-MEDIAN EMINENCE CELLS OF THE RAT HYPOTHALAMUS. HAMBA,M. and TODA,K. Dept.Physiol., Sch.Dent.,Showa Univ.,142 Tokyo. and Dept.Physiol., Fac.Dent., Tokyo Med. & Dent. Univ., 113 Tokyo.

Effects of parachlorophenylalanine (PCPA) on the neuronal activities of arcuate (ARH) cells which project to the median eminence and on analgesia induced by electro-acupuncture (EA) stimulation at different frequencies were investigated in Wistar albino rats. PCPA-treated or non-treated rats were anesthetized with thiamylal sodium (60 mg/Kg., I.P.). EA stimulation was delivered at 3, 45, 100 Hz (5 ms-duration, 300-500 uA) for 15 min to a meridian point Ho-Ku. After EA, three types of neurons were seen in ARH cells. TYPE I showed an increase in spontaneous firing rate and the response to noxious stimuli, TYPE II showed a decrease in both, and TYPE III showed no change. These excitatory or inhibitory effects of EA were reversed by naloxone (10 μg / μl) administered intraventricularly. In PCPA-non-treated rats, EA stimulation at 3, 45 or 100 Hz frequency produced a good suppressive effect on the jaw opening reflex, and the higher the frequency was, the more the numbers of TYPE II neurons were observed. In PCPA-treated rats, EA at higher frequency scarcely produced the analgesic effects, and the number of TYPE II neuron was decreased.

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ROLE OF ARCUATE NUCLEUS IN ACUPUNCTURE ANALGESIA AND NON-ACUPUNCTURE POINT STIMULATION-PRODUCED ANALGESIA. SATO, T and TAKESHIGE, C. Dept. of Physiology, Sch. of Medicine, Showa University, Shinagawa-ku, Tokyo 142

Since hypophysectomy abolished acupuncture analgesia (AA), the pathway from acupuncture point to hypophysis was defined as an acupuncture afferent pathway and the descending pain inhibitory systems activated by neurohumoral factors from hypophysis was defined as acupuncture efferent pathway. Lesion of the caudal arcuate nucleus (AN) abolished AA and 0.5mg/kg intraperitoneal morphine analgesia measured by tail-flick test of rat. Unlike acupuncture afferent pathway stimulation-produced analgesia, AN stimulation-produced analgesia (AN-SPA) was observed only during stimulation, did not show any individual variation in effectiveness and was not affected by naloxone nor dexamethasone, but completely blocked by dopamine antagonist, pimozide. AN-SPA was partially reduced by serotonin antagonist, methysergide and lesion of the ventral periaqueductal central gray containing the dorsal raphe nucleus and was completely blocked by the spinal dorsolateral funiculus lesion. Non-acupuncture point SPA revealed after lesion of the lateral centromedian nucleus of thalamus was also blocked by AN lesion. These results indicate that AN plays some role as common efferent pathway of AA and non-acupuncture point SPA, but what neurohumoral factors from hypophysis activate AN neurons is still obscure.

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NATURE OF NON-ACUPUNCTURE-POINT STIMULATION PRODUCED ANALGESIA AFTER LESION OF ANALGESIA INHIBITORY SYSTEM. KOBORI M., MERA H. and TAKESHIGE C. Dept. of Physiology, School of Medicine, Showa University, Tokyo 142

Augemented acupuncture point stimulation-produced analgesia(AA),measured by tail-flick latency of rats, after lesion of the lateral centromedian nucleus of thalamus(l-CM) was antagonized by dexamethasone. AA after lesion of the l-CM and the lateral septum(l-SP), which had been identified as AA producing system(R_2), was partially antagonized by dexamethasone. Remained analgesia after dexamethasone was antagonized by intraperitoneal naloxone(1 mg/kg). Non-acupuncture point stimulation did not produce any analgesia, however analgesia(NAA) was induced after lesion of l-CM by stimulation of non-acupuncture point with same stimulus intensity and frequency for AA. NAA was completely antagonized by dexamethasone. Analgesia produced by stimulation of AA afferent pathway(R_2) from acupuncture point to hypophysis was not antagonized by dexamethasone but by naloxone, while that of the pathway(R_1) inhibited by analgesia inhibitory system such as l-CM was partially antagonized by dexamethasone. Individual variation in effectiveness was observed in AA while was not observed in NAA. From these results it was concluded that central pathways are different in naloxone reversal AA from dexamethasone reversal NAA.

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PROPERTIES OF SUGAR BINDING SITE IN THE RAT TASTE CELL EXAMINED FROM NEURAL RESPONSES TO VARIOUS SUGARS. IMOTO, T., YAMADA, H. and HIJI, Y. Dept. of Physiology, Tottori University School of Medicine, Yonago 683

Present experiments were performed to investigate whether various sugars elicit the taste responses by interacting with the common binding site in the sugar receptor. By the treatment of the rat tongue with either dithiothreitole or Woodward's reagent K, the integrated responses to sucrose and fructose recorded from the chorda tympani were suppressed preferentially without any significant effect on those to glucose and maltose. The analyses of concentration-response relations obtained at various pH's also revealed that the apparent dissociation constants underwent changes similarly with pH in both glucose and maltose, while those for sucrose and fructose were almost constant over the wide range of pH (4.0-9.5). These results seem to be explained well by assuming that multiple binding sites are involved in the sugar receptor of the rat, each of which has an affinity to the sugar molecule characteristic to its molecular structure.

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ELECTRICAL PROPERTIES OF CHEMICALLY INDUCED POTENTIAL OF THE BULLFROG TONGUE. SOEDA, H., NODA, K., SAMEJIMA, C. AND SAKUDO, F., Dept. of Physiol., Fukuoka Dent. Coll., Sawara-ku, Fukuoka

As previously revealed, application of such chemicals as four basic taste substances can produced a slow potential on dorsal surface of the isolated bullfrog tongue (surface potential) from which longitudinal muscles are dissected out. Electric resistance between dorsal and the other side of preparation during the surface potential was decreased in NaCl-, acetic acid- and quinine-induced potentials, but increased in sucrose-induced potential. The change in resistance was dependent on potential amplitude as well as stimulus concentration. Amplitude of surface potential was decreased or reversed under conditioning cathodal polarization and increased under anodal polarization in NaCl-, acetic acid- and quinine-induced potential, but vice versa in sucrose-induced potential. Surface potential could also be produced on the tongue of anesthetized frogs and it's magnitude was proportional to afferent nerve activity recorded simultaneously. The results indicate that the surface potential is not simply physicochemical potential occurring at fluid interphase but has similarity to receptor potential in taste cells.

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EFFECTS OF TRANSECTION OF THE GLOSSOPHARYNGEAL NERVE ON FROG TASTE CELLS.
HONDA, E. AND NAKAHARA, S. Dept. of Physiol., Kyushu Dental Coll., Kitakyushu

In the mammal and fish, it is well known that taste buds are degenerated and disappeared after denervation. But we observed with microscope that taste cells of the frog were still maintained morphologically at 120 days after transection of the glossopharyngeal nerve.

Responses to four basic taste stimuli and resting potentials in 154 denervated taste cells at intervals of from 5 to 140 days after nerve section were recorded with a glass pipette micro-electrode. Depolarization produced by various chemical stimuli as the same as normal taste cells. The average value of receptor potentials to 0.5 M NaCl, 0.01 M HCl and 0.02 M quinine-HCl were 10.5±2.5 mV (7 days) and 1.5±0.9 mV (140 days), 14.4±5.2 mV (7 days) and 13.0±16.9 mV (140 days), and 1.4 ±2.0 mV (7 days) and 6.0±2.2 mV (105 days) respectively. The transection of the glossopharyngeal nerve had no significant effects on resting potentials of denervated taste cells.

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The role of Ca ions in receptor potential of frog taste cell in response to acid stimuli.
OKADA, Y. and SATO, T. Dept. of Physiol., Nagasaki Univ. Sch. of Dent., Nagasaki, 852

The role of Ca^{2+} ions in the acid-induced receptor potential of frog taste cells was studied. Acetic and hydrochloric acids in 0.1 M NaCl or deionized water (D. W.) were used for taste stimuli. The amplitudes of receptor potentials produced by acids scarcely changed even when Na^{+} and Ca^{2+} ions in the intercellular fluid sorrounding taste cells were removed. The receptor potential amplitude for HCl dissolved in D. W. decreased to 70% of the control when Na^{+} ions in the adapting solution covering the receptor membrane surface were replaced with choline. The receptor potential amplitude also changed depending on a concentration of Ca^{2+} ions in the adapting solution. Addition of 5 mM Co^{2+} ions to the adapting solution decreased the receptor potential amplitude to 70% of the control. These results suggest that Ca^{2+} and Na^{+} ions may play an important role in the generation of taste receptor potential in response to acids.

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ION DEPENDENCE OF THE EEL TASTE RESPONSE TO AMINO ACIDS.
YOSHII, K. and KURIHARA, K. Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060.

The effects of changed ionic environments on the eel taste responses to amino acids were examined by recording the activity of the palatine nerve. 1. The responses to glycine, L-proline, and L-arginine dissolved in deionized water were greatly reduced after ions were removed from the surface of the palatine epithelium by the treatment with 5 mM EDTA. The addition of various species of salts to amino acid solutions reversibly recovered the responses. 2. The effects of salts of divalent cations ($CaCl_2$ and $MgCl_2$) appeared at much lower concentration (10^{-7}M) than those of salts of monovalent cations (NaCl and KCl)(10^{-4}M), suggesting that cations support the taste response. 3. All the organic cations examined including those of large molecular weights (choline, Tris, D-glucosamine etc) also supported the taste response. 4. The results obtained led to a conculsion that not a specific cation but various species of cations can support the eel taste responses. It is discussed that the cations do not act as current carrying ions to depolarize the taste cells but the binding of the cations on the receptor membrane plays an essential role in the taste reception. The present results were essentially similar to those obtained with the olfactory responses of the fishes and the frog (Brain Res. in press), which suggests that there exist a common transduction mechanism in taste and olfactory cells.

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DECLINE OF CHORDA TYMPANI TASTE RESPONSES IN THE RAT AFTER NERVE TRANSECTION. KITADA, Y. Dept. of Physiology, Okayama University Dental School, Shikata-cho, Okayama 700

The chorda tympani nerve (CT) in the anesthetized rat was exposed, and cut near its entrance into the tympanic bulla. Neural taste responses to stimulation of the tongue with 0.1 M NaCl were recorded from the CT. Changes in magnitude of taste response with time after cutting the CT were investigated. Taste responses were stable for 8-10 hours after CT transection. The taste response declined to 50% of the initial response at 13 hours and to 10% at 16 hours after transection. To test viability of the nerve trunk the distal portion of the CT was electrically stimulated. The magnitude of taste responses and the amplitude of the compound action potential evoked by the electrical stimulation declined in parallel. After decline in taste response, the nerve was dissected more distally, and reset again on the electrode. The procedure leads to longer survival of the taste response. These results indicate that the proximal portion, near the cut end, deteriorates with time and the distal portion still survives. Since the length of the distal stump of the rat CT is long, it is likely that the deterioration of the nerve at the recording site occurs faster than the depletion of axonal transport substances which have trophic effects upon taste buds.

This work was done at Dept. of Oral Biology, School of Dentistry, University of Michigan. I thank Dr. R. M. Bradley and Dr. C. M. Mistretta.

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GUSTATORY CROSS-ADAPTATION EXPERIMENTS FOR AMINO ACIDS IN THE CARP. MARUI, T., HARADA, S. and KASAHARA, Y. Dept. Oral Physiol., Kagashima Univ., Dent. Sch., Kagoshima 890

The importance of amino acids as olfactory and taste stimuli for fishes has generally been recognized. Previous electrophysiological studies determined that receptors of the facial taste system of the carp are highly responsive to L-α-amino acids containing three to four carbon atoms and the threshold concentration for L-Pro, the most effective amino acids tested, ranged between 10 and 1 nM. Also, it is suggested from the study of stimulatory effectiveness for amino acids' derivatives that stimulatory effectiveness of amino acids depends on their interactions with receptor membrane structure of difinite shape, size and probably charge distribution. In the present electrophysiological study, cross-adaptation experiments were performed to clarify interactions with more than one type of receptor site and lead to a postulate that a multiplicity of gustatory receptor types exist in the carp; L-Ala, L-Pro, L-Glu Na, L-CysH, Betaine, or L-Asp Na at 1 mM which are stimulatory on the gustatory receptors was utilized as a constantly applied adapting stimulus, whereas the test stimuli consisted of 1 mM applications of the other effective amino acids dissolved in 1 mM adapting solutions. Integrated activity from whole nerve recordings shows that Betaine and L-Asp Na enhance the activity for almost all of other amino acids. Some amino acids hold their receptor subsites in common, while each of L-His, L-Asp Na and Betaine is independent of subsites of the other chemicals.

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TASTE RESPONSE TO AMINO ACIDS FROM CHORDA TYMPANI NERVES IN MOUSE AND RAT HARADA, S., MARUI, T. and KASAHARA, Y. Dept. of Oral Physiol., Kagoshima Univ. Dent. Sch., Usuki-cho, Kagoshima 890

The taste responses to 18 amino acids at 0.1M were recorded from chorda tympani nerves in mouse and rat. The most effective amino acids were Arg-HCl and Lys-HCl in both animals, and the pattern of integrated responses and dose-response curves for them were identified almost the same as those for NaCl. Though there was no significant difference between the responses to L- and D-Arg-HCl, the responses to D-forms of Ser and Ala were significantly smaller than those to their L-forms. When the tongues were adapted to basic amino acids or NaCl, following responses to NaCl or basic amino acids were almost eliminated. Applications of a few derivatives of Arg and Lys revealed that amino group concernes to their effectiveness for taste stimulation. The responses to free basic amino acids (His and Lys at 0.1M) were quite smaller than that to 0.1M NaCl, and larger responses like NaCl were emerged when HCl or H_2SO_4 was added to those amino acids solutions. These results suggests that the charge of the amino group in the basic amino acids is essential for the stimulatory effectiveness similar to that for NaCl.

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INTERSTRAIN DIFFERENCES IN TASTE RESPONSES IN MICE.
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Behavioral investigation has revealed that SWR/J mice avoided a 10^{-4} M bitter taste solution of sucrose octaacetate (SOA) whereas other strains did not (Lush, 1981). Harder and Whitney (Dept. Psychol., Florida State Univ.) have obtained almost the same results. In the present experiment electrophysiological studies were performed in order to obtain a neurophysiological basis of the behavioral difference of drinking. Neural responses were recorded from the glossopharyngeal and chorda tympani nerves. Animals were adult male mice of SWR/J, LP/J, BDP/J and DBA/2J strains. The results showed a big difference in the neural response to SOA between SWR/J and the other strains. SOA solutions elicited a marked response in SWR/J. The threshold was between 10^{-5} M and 10^{-7} M. On the other hand, the solution produced a very small response even at concentrations of 10^{-4} M and 10^{-3} M in the other strain mice. There were no appreciable differences in the taste response produced by other test solutions (QHCl, PTC, NaCl) among the strains.

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GUTATORY RESPONSES TO AMINO ACIDS IN MICE. IWASAKI, K., KASAHARA, T.* and SATO, M. Department of Neurophysiology, Tokyo Metropol. Instit. for Neurosci., Fuchu, Tokyo

Neural and behavioral responses to L- and D-amino acids in the ddy mouse were studied by recording the integrated nerve discharges from the chorda tympani and using the two-bottle preference method. L-Amino acids with side chain containing basic group and HCl showed high neural response magnitudes and low thresholds compared with those for other amino acids. Among the latter groups the neural response magnitude decreased with increasing M.W. and hydrophobicity. Mice preferred L-amino acids that taste sweet to humans, while they rejected those which taste bitter. There was a significant negative correlation between the preference ratio and M.W. as well as hydrophobicity, indicating that these parameters determine the taste of the L-amino acids. Mice preferred D-Ala, -Phe and -Met, though L-Phe and -Met were rejected. The neural response magnitude for L-isomers was generally larger that that for D-isomers. There was a good agreement between the preference for L-amino acids by the mouse and that by the rat, and the former agrees with the hedonic intensity produced by amino acids in humans.

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EFFECTS OF RESECTION OF TASTE NERVES ON SOLUTION INTAKE IN RATS. YAMAMOTO, T., ASAI, K. and KAWAMURA, Y. Dept. of Oral Physiology, Osaka University Dental School, Kita-ku, Osaka 530

Effects of resection of the taste nerves on liquid licking behavior were examined. Wistar male rats were trained to drink solutions in a test box. The number of licks / 20 sec was monitored using a drinkometer for each test solution such as 0.5M sucrose, 0.01 - 1.0M NaCl, 0.03M HCl and 0.0005 - 0.01M quinine-HCl. The 3 taste nerves, the anterior palatal, chorda tympani (CT) and glossopharyngeal (GL) nerves, were cut bilaterally in various combinations. Rats without one of the 3 taste nerves still rejected the aversive HCl and quinine solutions. However, after resection of both CT and GL or all of the 3 nerves, the rats showed a significant increase in the number of licks to these aversive solutions in comparison to normal rats. For the palatable solutions, the interlick interval, lick duration and the amount of intake per each lick did not change significantly after resection of taste nerves in any combinations. Considering the number of taste buds innervated by each taste nerve, the present results suggest that taste aversive behavior in rats occurs even after removal of about 80% of total taste buds.

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GUSTATORY NEURAL CODING IN THE MOUSE.
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Responses of single chorda tympani fibers of the mouse to 13 taste stimuli were compared to behavioral responses to these compounds. Animals were given a conditioned taste aversion to one of the 13 stimuli and were tested for generalization of the learned aversion. Cross-aversion to the 13 stimuli showed that mice discriminate not only among the 4 basic taste stimuli but also among different sweeteners, especially between maltose and other sugars and saccharin Na. The across-fiber correlation in the firing rates evoked by these stimuli in the chorda tympani corresponded well to behavioral responses to acids, QHCl and salts without NaCl, but not to those to the sweeteners and NaCl. Fibers responding best to the sweeteners or NaCl were narrowly responsive, even when the other taste stimuli were very intense, and account well for behavioral responses to these stimuli. These results suggest the possibility that mice possess a double-coding system of taste quality; one is coding by the pattern of activity across many neurons, and the other is more dominant coding than the former by specific taste quality lines formed by some of the neurons.

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PROJECTIONS OF THE PARABRACHIAL TASTE NEURONS TO THE THALAMUS AND THE AMYGDALA. HAYAMA, T., ITO, S., AND OGAWA, H. Dept. of Physiol. Kumamoto Univ., Med. Sch., Kumamoto, 860.

We examined the projections of the 68 parabrachial (PB) taste neurons to the bilateral thalamic taste areas (TTA) and the ipsilateral central nucleus of the amygdala (CA) in barbiturate-anesthetized rats. Of the 55 units tested for TTA projection, 33 were excited antidromically (P-T), and 8 of the 31 units tested for CA projection were invaded (P-A). Of the 18 units tested for both projections, we identified 12 P-T units, 4 of which were also P-A units, and one P-A unit of no connection to the TTA. Antidromic latencies (L) of the P-T units to ipsilateral TTA stimulation were shorter than those of the P-A units to CA stimulation. Ls to ipsilateral TTA stimulation were not different between the P-T units with and without the axon collaterals to the CA. The P-T units produced larger mean magnitudes of responses to all of the 4 basic taste stimuli than the P-A units. Most of the P-T units were excited best by hedonically positive taste stimuli among the 4 basic stimuli, but most of the P-A units by hedonically negative ones. It was suggested that the PB may send hedonically differential taste information to the TTA and CA.

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CORTICAL TASTE NERVE PROJECTION AREA IN THE MACAQUE AS REVEALED BY A FIELD POTENTIAL ANALYSIS. ITO, S., NOMURA, T., and OGAWA, H. Dept. of Physiol. Kumamoto Univ. Med. Sch., Honjo 2-2-1, Kumamoto, 860

To identify the cortical gustatory area in the macaque, the ipsilateral taste nerve projection areas were located. The evoked potentials were recorded by a gross tungsten electrode from the buried cortices in the lateral fissure (LF) of eight nembutal-anesthetized Japanese macaques while stimulating the lingual (L), chorda tympni (CT), and glossophalyngeal (G) nerves. Responses to L stimulation were small and confined to the operculum. CT potentials were found at three focal sites falling between the beginning of the LF and that of the anterior subcentral sulcus: (1) the dorsalmost operculum and its transitional zone to the insular cortex across the circular sulcus, (2) the lip of the dorsal operculum, and (3) the ventral insular cortex. The largest responses were always recorded at the former site among others. G-responsive areas largely overlapped with those of the CT, but the G response was smaller than CT response at the dorsal operculum. The present findings suggest that the dorsal opercular cortex is a most likely candidate for the gustatory cortex.

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RELATIONS AMONG FLOW RATE, pH AND THE CONCENTRATIONS OF Na^+, Cl^-, HCO_3^-, Ca^{2+},K^+, INORGANIC P IN HUMAN PAROTID SALIVA. INOMATA, K., TAMAGAWA, K., ITO, K., NAKAMURA, H*. and KURAHASHI, M*., Dept of Physiol. and Oral Physiol*., Higashi-Nippon-Gakuen Univ., School of Dent., Ishikari-Tobetsu, Hokkaido, 061-02

After tongue was stimulated by 0.5 ml of 3% tartaric acid, the flow rate of parotid saliva was increased. Maximum flow rate was registered 20 seconds after stimulation, and 5 minutes later, this flow rate decreased to the prestimulus level. Prestimulus saliva pH was 6.5. One minute after stimulation, saliva pH increased to 7.5, and this value was maintained for about 3 minutes, but after that, the pH decreased to the prestimulus level. Maximum concentrations of saliva Na^+ and Cl^- were registered about 20 seconds after stimulation (Na^+: ca 60, Cl^-: ca 50 mEq/l), and later, both concentrations decreased gradually. Maximum concentration of saliva HCO_3^- was registered 30 seconds after stimulation. After that, the concentration of saliva HCO_3^- decreased gradually, and it returned to the prestimulus level 8 minutes after stimulation. The concentration of saliva Ca^{2+} did not show any changes after stimulation (2 mEq/l). Both concentrations of saliva K^+ and inorganic P decreased after stimulation, and minimum concentrations of saliva K^+ and inorganic P were registered 1 minute and 20 seconds respectively after stimulation. The time courses of the concentrations of all these ions above shown did not show the same pattern.

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NEUROBLASTOMA CELL AS A MODEL FOR OLFACTORY CELL AND TASTE CELL:MECHANISM OF DEPOLARIZATION IN RESPONSE TO "ODORANTS" AND "BITTER SUBSTANCES". KASHIWAYANAGI M.*, KUMAZAWA T.* AND KURIHARA K. Fac. of Pharm. Sci., Hokkaido Univ., Kita-ku, Sapporo 060

The mouse neuroblastoma cell(N-18 cell) was used as a model for olfactory cell and taste cell and the mechanism of depolarization in response to "odorants" and "bitter substances" was studied. The membrane potential and the membrane resistance were measured by the conventional intracellular technique. 1) "Odorants"(n-amyl acetate, camphor, β-ionone, vanillin, ethanol and tetrahydrofuran) and "bitter substances" (quinine, papaverine, indole, nicotine and bacitracin) dissolved in Ringer solution reversibly depolarized the N-18 cell. 2) The depolarization induced by the "odorants" and the "bitter substances"(except quinine) was not accompanied appreciable changes in the membrane resistance. 3) "Odorants"(5 mM n-amyl acetate and 0.1 mM β-ionone) and "bitter substances"(0.1 mM papaverine and 2 mM indole) in Na-free solution, Cl-free solution and low Ca-solution induced depolarization similar to those in normal Ringer solution. Results obtained suggest that depolarization of N-18 cell in response to "odorants" and "bitter substances" is induced by changes in the phase-boundary potential.

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EXTERNAL CALCIUM CONCENTRATIONS ON THE DISCHARGE FREQUENCY RESPONSES OF THE AESTHETASC NEURON. CHICHIBU, S., KITAGAWA, C. and CHIBA, A. Dept. Physiol., Kinki Univ. Sch. Med., Sayama-cho, Osaka 589.

Impulse discharges of the crayfish aesthetasc neurons were recorded with a pair of German-silver needle electrode externally. Impulse frequencies were calculated on every one second of discharge series. Comparisons of impulse frequency changes were made on normalized discharge frequencies with the control discharges. These neurons were sensitive to several amino acids including glycine. Decay of the discharge ratio due to the adaptation was similarly exponential, though the higher calcium concentration produced higher instantaneous discharge ratio. The adaptation for glycine was completed within 10 min. When the concentration of glycine was fixed at 10 mM and the calcium concentration was changed from 0.1 to 50 mM, the discharge ratio showed an increase at the concentrations of 3-20 mM Ca. By changing of the stimulus concentrations of glycine and of calcium within the ranges of 0.1 to 50 mM, a two-dimensional discharge ratio diagram was obtained. The combination of Gly and Ca produced the highest discharge was the ranges of 1.6 to 12.5 and of 5 to 20 mM, respectively. This Ca concentration is very close to that contained in the van Harreveld solution, and the higher Ca concentration over 13.5 mM is related to the sensitivity of the aesthetasc neuron.

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COMPARISON OF THE DISTRIBUTION PATTERN OF THE DENDRITES AND AXONS BETWEEN MITRAL AND TUFTED CELLS. MORI, K., *KISHI, K. and *OJIMA, H. Dept. of Physiol. Gunma University School of Medicine, Maebashi, Gunma 371 and *Dept. of Anatomy, School of Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113.

To determine the laminar organization of the dendrites and local axon collaterals, mitral, displaced mitral and middle tufted cells in the rabbit olfactory bulb were stained by intracellular injection of HRP. The secondary dendrites of mitral cells were distributed mostly in the inner half of the EPL while their local axon collaterals were distributed widely in the GrL. Displaced mitral cells projected their secondary dendrites mainly in the middle and superficial sublayers of the EPL. The projection pattern of the local axon collaterals of displaced mitral cells was similar to that of mitral cells. The secondary dendrites of middle tufted cells were distributed mostly in the superficial portion of the EPL whereas their local axon collaterals were distributed mainly in the superficial sublayers in the GrL. The differential ramifications of dendrites and local axon collaterals suggest the functional differentiation among mitral, displaced mitral and middle tufted cells.

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OLFACTORY PROJECTIONS THROUGH SUBCORTICAL RELAYS IN CATS AND RABBITS. OHTA, N. and MOTOKIZAWA, F. Dept. of Physiol., Nara Medical College, Kashihara, Nara

A cortico-cortical olfactory projection has been reported in the cat (Motokizawa and Ino, 1981). In the present experiment, olfactory projections via subcortical relays were investigated using the retrograde transport of horseradish peroxidase(HRP) and by means of the electrophysiological technique. The animals were anesthetized with sodium pentobarbital. After injection of 0.05 to 0.1 µl of a 50% solution of HRP in the physiological saline into the mediodorsal thalamic nucleus(MD), labeled cells were found in the g.proreus, g.sigmoideus, g.cinguli, insular cortex, olfactory tubercle, claustrum and hypothalamus. Among these structures, the insular cortex and hypothalamus are known to receive afferents from the piriform cortex(PC). This indicates that PC projects to MD indirectly, coinciding the previous electrophysiological finding (Motokizawa, 1974). When HRP was injected into the orbitofrontal cortex(OF), labeled cells were found in the g.cinguli, ventral anterior, ventral medial and mediodorsal nuclei of the thalamus and the deep amygdaloid nuclei(AMYGd). Cell labelings in AMYGd following injection of HRP into OF were obtained also in the rabbit. Orthodromic unit firing was elicited following stimulation of both MD and AMYGd in the cat. Since AMYGd connects with PC multisynaptically, there are two subcortical routes from PC to OF; one via MD, the other via AMYGd.

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FUNCTIONAL ARCHITECHTURES OF SENSORY NERVE TERMINAL IN THE FROG MUSCLE SPINDLE. FUJITSUKA, N. and ITO, F. Dept. of Physiology, Nagoya University School of Medicine, Showa-ku, Nagoya 466

The present study was made to define the site of mechanical transduction along the non-myelinated nerve terminal of the frog muscle spindle, using an ordinal and high voltage (1000KV) electron microscope. The muscle spindle was isolated from sartorius and semitendinosus muscles and freezed quickly in either slacked or stretched state. The specimens were incubated in cooled acetone with 4 % osmic acid. Thin cross and thick (5µm) longitudinal sections were made semi-serially. The slack spindle was characterized by a loose connection between the nerve terminals and intrafusal muscle fibers in the reticular zone. In stretched state, the diameters of intrafusal muscle fibers and the non-myelinated thin threads along the reticular zone decayed almost half, but insignificant changes were found in those diameters along the compact zone.

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ELECTROGENIC ION PUMP IN THE SENSORY NERVE TERMINAL OF FROG MUSCLE SPINDLE. ITO,F., FUJITSUKA,N., KOMATSU,Y. and KANEKO,N. Dep.Physiol. Nagoya Univ. Sch. Med., Nagoya 466.

Muscle spindles were isolated from frog sartorius and semitendinosus muscles. Individual afferent spikes recorded from the sensory nerve terminal by means of an air-gap method were followed by an after hyperpolarization (AHP). The amplitude of the AHP was reduced by application of K^+ and Ca^{2+} channel blockers, but a small residue of the AHP was still remained. The residue was erased by treatment of one of methabolic inhibitors (1 μM carbonyl cyanide m-chlorophenyl hydrazone, 10 μM ouabain, or 100 μM N-ethylmaleimide). The amplitude of the residue was sensitive to polarizing currents. While the amplitude of the individual afferent spikes was increased by decreasing fluid temperature from 34 to 12 °C, the amplitude of the residue was decreased by the temperature decay. These results suggest that the residue is due to an electrogenic ion pump.

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CHARACTERISTICS OF THE TWO-POINT FUSED SENSORY AREA OF THE SKIN. IWASE.Y.,MAEYAMA.F* and KUMAMOTO.K Dept. of Physiology, Meiji College of Acupuncture and Moxibustion, Hiyoshi-cho, Funai-gun, Kyoto 629-03

Applying the two points of touch and pressure stimuli to the skin, the threshold separation required for the two stimuli to be noticed as one rather than two points was determined. On the same region of the body, three longitudinally combined points and one transversally combined point were measured and the area which was surrounded by them was cal culated. It was called two-point fused sensory area (fused area), and tested on the limbs and the trunk. Moreover, the fused area is smaller at the peripheral part than the central one on the same dermatome. These results are not concerned with the pressure point density of the skin. On the same region, the area enlarges along the axis, and is more enlarged through the touch stimuli than through the pressure ones. Acupuncture stimuli enlarges the area, too.

Some deductions, concerning the characteristics of the two-point fused sensory area, were made, that the enlargement of the area is referring rather to the central nervous mechanisms, lateral inhibition.

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A JAW CLOSING REFLEX EVOKED BY WATER STIMULATION ON THE ORAL MUCOSA OF THE FROG. NOMURA, H. and KUMAI, T. Dept. of Oral Physiology, Matsumoto Dental College, Shiojiri 399-07

The specific chemoreceptor sensitive to tap water in the frog was suggested to play a role in keeping the mouth reflexly closed (Zotterman, 1949). So far as we had studied, however, the water on the tongue elicited reflex discharges in the nerves innervating the nostril closing muscles, but not in the nerves innervating the jaw closing muscles. Nevertheless, we thought that the frog should have a jaw closing reflex mechanism, because the frog is known to keep its mouth closed in the water. Male frogs, Rana nigromaculate and Rana japonica, were used. The activities of the temporal and pterygoid muscles were examined by recording EMG, while that of the masseter muscle by recording neural discharges. The latter was used for measuring the latencies of the reflex. The chemoreceptor of the oral mucosa were found to be innervated by the facial nerve; internal maxillary and palatal nerves. The water on the oral mucosa elicited afferent neural discharges in these nerves and reflex discharges in the nerves innervating the masseter and pterygoid muscles. The area around the inner nostril was found to be highly sensitive to chemical as well as tactile stimulation. This suggests that a water flow though the nostril elicits a jaw closing reflex.

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STRUCTURE AND FUNCTION OF SLOWLY ADAPTING PACINIAN CORPUSCLES IN THE ADULT CAT'S TOES.

SAKADA, S., YAMAMOTO, T., TAZAKI, M. and SHIOZAKI, K.
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It is generally known that Pacinian corpuscles are rapidly adapting mechanoreceptors. Also, it has been considered that the mechanical filter action of the Pacinian corpuscle makes this transciet response i. e. rapid adaptivity. In our experiment by separating a single nerve fiber supplying the corpuscle and giving pressure stimulation, however, the adaptation times of the responses from the nerve fibers clearly indicated that there were two kinds of the adult cat's corpuscles, the fast-adapting type and the slow-adapting type. Furthermore, according to the electron microscopic findings, whereas there was similarity in the inner cores of both kinds of Pacinian corpuscles, great difference was observable in the arranged lamellae of their outer cores. that is, whereas the number of the lamellae was ordinarily 30 or more in the fast-adapting type, they were only 20～23 in the slow-adapting type. In addition to the characteristics of the response of slow-adapting type Pacinian corpuscles, those to ramp and vibratory stimuli will be discussed in this session.

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POST-CONTRACTILE ATP UTILIZATION IN SKELETAL MUSCLE AS REVEALED BY TIME-RESOLVED ^{31}P NMR. TANOKURA, M. and YAMADA, K. Dept. of Physiol., Medical College of Oita, Oita

The course of changes of the creatine phosphate (PCr) and inorganic phosphate (P_i) after the various periods of isometric contraction was studied by ^{31}P nuclear magnetic resonance (NMR) with 8 pairs of toad sartorius muscles at the time-resolution of 16 s at 4°C. The results indicated a post-contractile PCr (or ATP) splitting; the level of PCr continues to decrease for a few min after the muscle has relaxed from tetanus, whereas P_i becomes maximal at the end of contraction and starts to decrease immediately after the contraction is over. The plot of the amount of the PCr splitting extrapolated to time zero (immediately after the relaxation) versus the period of contraction is rectilinear. The amount and the course of producing the post-contractile PCr splitting is similar to those of the unexplained heat production. The above results can be explained if almost all myosin molecules are in the actin-myosin ADP form (A-M·ADP) in 2 to 3 s after the stimulation has started. A-M·ADP has already released P_i, but returns slowly to the resting form of myosin-product complex (M·ADP·P_i) by consuming one molecule of ATP.

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THE ONSET OF CROSS-BRIDGE MOVEMENT AND THE ONSET OF CONTRACTION. MATSUBARA, I. and YAGI, N. Dept. of Pharmacology, Univ. of Tohoku Sch. of Medicine, Seiryo-machi, Sendai 980

The transfer of myosin heads from the thick to the thin filaments during twitch is known to proceed faster than tension development; at 0 °C, the molecular change reaches the maximum 90 msec before the peak tension. However, it is not clear whether the onset of the molecular change precedes, or coincides with, the onset of tension development. This study was carried out to clarify this point.

A sartorius muscle of the bullfrog was held at a sarcomere length of 2.5 μm and stimulated with single electrical pulses at 1 °C. The 1,0 and 1,1 equatorial X-ray reflections from the hexagonal myofilament lattice were recorded at a time resolution of 1 msec. The intensities of these reflections were used as indicators of the cross-bridge movement. The 1,0 intensity started to change 13.8 msec after the stimulus, and the 1,1 intensity 11.8 msec after the stimulus. These times corresponded approximately to the start of tension increase after the latency relaxation. Thus the onset of cross-bridge movement does coincide with the onset of tension development.

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EFFECTS OF CAFFEINE ON CROSS-BRIDGE MOVEMENTS DURING MUSCLE CONTRACTION. YAGI, N. and MATSUBARA, I. Dept. of Pharmacology, Univ. of Tohoku Sch. of Medicine, Seiryo-machi, Sendai 980

Effects of caffeine on the behaviour of myosin heads during twitch were studied by the X-ray diffraction method. A sartorius muscle of the bullfrog was held isometrically in Ringer's solution (15 °C) and stimulated with single electrical pulses. The duration of each twitch was divided into 14 periods of 12.5 msec. The number of myosin heads associated with actin was estimated for each period, based on the 1,0 and 1,1 equatorial reflections. The number increased as the tension developed during twitch. In the absence of caffeine the number reached a maximum 25 msec after stimulus. The maximum number was 60% of all myosin heads, smaller than the value obtained during maximum tetanus (76%). The number stayed maximum for 40-50 msec, before falling to the resting level on relaxation. Caffeine (1 mM) increased the twitch tension by 41%. This was accompanied by an increase in the number of heads associated with actin; the maximum was 77% of all myosin heads, almost the same as that during tetanus. These results were interpreted in relation to the old concept of the active state.

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THE EQUATORIAL X-RAY DIFFRACTION PATTERN FROM STRETCHED FROG SKELETAL MUSCLES.
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Changes in the equatorial X-ray diffraction pattern from tetanized frog sartorius muscles(Rana Catesbiana) were studied by use of time-resolved data collection technique (time resolution, 0.5 sec) to give information about properties of the cross-bridges. No significant changes in the intensity ratio of two equatorial reflections(I(1,0)/ I(1,1)) were observed when isometrically contracting muscles were slowly stretched by 5-6%, in spite of marked force changes. The intensity ratio also showed no significant changes when the load on isometrically contracting muscles were suddenly increased from P_o to 1.2-1.3 P_o to produce isotonic muscle lengthening. Closer examination of the data indicated that a small decrease in the value of I(1,1) was caused by both slow stretch and isotonic lengthening. Because of the scatter of the experimental plots in I(1,0), the effect of small changes in I(1,1) on the intensity ratio fell within the range of accuracy of measurement. Similar results were obtained when the muscles in rigor were stretched. It is suggested that no marked changes in myosin head orientation or in the number of the cross-bridges in the vicinity of thin filaments take place in response to slow stretches, isotonic lengthening and stretches of rigor muscles, and that the decreased regularity of the filament lattice may produce the change in I(1,1).

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FLUORESCENCE SPECTRA OF ANM-LABELED, GLYCERINATED RABBIT PSOAS MUSCLE FIBERS IN RIGOR, RELAXATION AND CONTRACTION.
CHAEN, S. AND SUGI, H. Central Lab. and Dept. of Physiol., Sch. of Med., Teikyo Univ. Tokyo.

Fluorescence spectra of ANM-labeled, glycerinated rabbit psoas muscle fibers were measured in rigor, relaxation and contraction. SDS polyacrylamide gel electrophoresis of ANM-labeled muscle fibers showed that myosin heavy chain was selectively labeled with ANM. Isometric tension development was not significantly influenced by ANM labeling. Increase in the fluorescence intensity and a blue shift of the emission maximum of the dye was observed during the transition from relaxation to contraction. During the transition from relaxation to rigor, there was a slight increase in the fluorescence intensity. These fluorescence changes were the same as that obtained from the ANM-labeled heavy meromyosin. The above results suggest that the fluorescence spectrum of ANM-labeled muscle fibers reflects a state of myosin in the actomyosin ATP hydrolysis cycle.

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RECONSIDERATION OF THE INDIRECT INTRACELLULAR Na AND K MEASUREMENTS IN THE RAT SKELETAL MUSCLES.
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The extracellular space, muscke Na and K contents and dry-to-wet weight ratio of skeletal muscles, which give the intracellular Na and K concentrations based on Desmedt's equation, were reconsidered using both soleus and extensor digitorum longus muscles of various aging rats such as young, adult and old. The measurements of extracellular space by inulin and muscle Na and K contents by flam photometer were followed by a considerable over estimation when the muscle weight as preparation was less than 50 mg. However, the error was markedly reduced in the muscle weight heavier than 80 mg. Consequently, it was concluded that the measurements of extracellular space and muscle Na and K concentrations should be performed at arround 80 to 100 mg total muscle weight by adding or cutting the muscle preparations from various aging rats. On these consideration, it was evident that younger rat muscles contained less intracellular Na concentration and much intracellular K concentration as compared with older rat ones.

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Ca^{2+}-INDUCED Ca^{2+} RELEASE FROM FRAGMENTED SARCOPLASMIC RETICULUM OF SKELETAL MUSCLE. KOSHITA, M. and HOTTA, K. Dept. of Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467

Release of Ca^{2+} from sarcoplasmic reticulum(SR) by external Ca^{2+} (Ca_o) was studied. Fragmented SR(FSR) prepared from bullfrog skeletal muscle was fractionated into light SR(LSR, derived from longitudinal tubules) and heavy SR(HSR, derived from terminal cisternae). $^{45}Ca^{2+}$ was actively taken up by FSR and was released by 20-fold dilution with a solution of various concentrations of Ca_o. Ca^{2+} release from FSR was accelerated and decelerated by the presence of micromolar and millimolar concentrations of Ca_o, respectively. Millimolar concentrations of Mg_o inhibited Ca^{2+} release at 10^{-5}M Ca_o, but no effects were observed at 10^{-8}M or 10^{-2}M Ca_o. Ca^{2+} efflux was enhanced by ATP in the medium at any concentrations of Ca_o. The dependence of Ca^{2+} release on Ca_o was not affected by the amount of Ca^{2+} loaded in FSR. Acceleration and deceleration of Ca^{2+} release by Ca_o were also seen in LSR and HSR. The possible role of Ca^{2+}-induced Ca^{2+} release in E-C coupling is postulated by comparing with that observed in skinned muscle fiber.

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ON THE RECOVERY OF Ca SENSITIVITY IN Ca INSENSITIVE GLYCERINATED MUSCLE FIBER. WATANABE, Y., NAKAYAMA, Y., WATANABE, K* and YAMAGUCHI, M** Dept. of Physiol. and Anat., Kyoritsu Coll. of Pharm., Minato-ku, Tokyo 105. *Dept. of Biol., Coll. of Science and Technol., Nihon Univ., Funabashi, Chiba 274, **Dept. of Biochem. and Nutri., School of Physical Education, Juntendo Univ., Narashino, Chiba 275

Ca insensitive glycerinated muscle fiber (CaIS-fiber) produced tension with only addition of ATP in the absence of Ca^{2+}, chelated with EGTA. This fiber may be made by the digestion of Ca regulatory protein, troponin tropomyosin complex. We attempted to elucidate this point with using tension mechanogram and SDS polyacrylamide gel electrophoresis. Electrophoretic pattern of CaIS-fiber actually proved that the troponin tropomyosin complex was going to be faint by digestion of trypsin or of intrinsic proteolytic enzyme and that a new band appeared at 30 K daltons peptide as the digested product of the regulatory protein. Furthermore, CaIS-fiber recovered Ca sensitivity on the tension and SDS gel pattern with incubation in native tropomyosin from skeletal muscle for 48 hours at 5°C. That is to say, the recovered fiber produced tension with addition of Ca^{2+} in the presence of ATP and appeared thick band at the position of regulatory protein on gel. It was presumed that the recovery of Ca sensitivity was due to the mending of digested protein of CaIS-fiber.

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EFFECTS OF EXTERNAL CALCIUM REDUCTION ON BIPHASIC POTASSIUM CONTRACTURES AND ACTION OF DIVALENT CATIONS ON THE EFFECTS IN FROG SINGLE TWITCH MUSCLE FIBERS. TAKAUJI, M., TSUTSU-URA, M. AND KANAYA, H. Dept. of Physiol., Sapporo Med. College, Sapporo

We examined effects of Mg^{++} and Ni^{++} on the initial component and the secondary component of 80 mM K contractures in the absence of Ca^{++} in frog single twitch muscle fibers. The peak tension of the initial component was potentiated by Ca reduction for 30 sec. This potentiation was not observed in the presence of 3 mM Mg^{++} or 0.5 mM Ni^{++} and in each case the peak tension of the initial component reached the control value obtained at 1.8 mM Ca. Thus, both 3 mM Mg^{++} and 0.5 mM Ni^{++} can completely substitute for 1.8 mM Ca^{++} in developing tension of the initial component. On the other hand, the peak tension of the initial component was inhibited by Ca reduction for 20 min and this inhibition was suppressed in the presence of 3 mM Mg^{++}. These results may be explained by stabilizing actions of these cations on fiber membranes. In contrast to this, there was a clear difference in the Mg^{++} effect and the Ni^{++} effect on the secondary component; the inhibition, induced by the reduction of external Ca^{++}, of the secondary component was only partially removed by 3 mM Mg^{++}, whereas it was almost completely removed by 0.5 mM Ni^{++}. On the basis of these results, the reason of the difference between the Mg^{++} effect and the Ni^{++} effect on the secondary components was discussed.

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INTRACELLULAR Ca^{++} TRANSIENT IN RAPID COOLING CONTRACTURE OF SINGLE SKELETAL MUSCLE FIBRES. KURIHARA, S., KONISHI, M. and SAKAI, T. Dept. of Physiology, Jikei Univ. Sch. of Med., Minato-ku, Tokyo 105 Japan

Rapid lowering the bathing solution temperature from 18 to 3°C induces contracture in skeletal muscle fibres treated with low concentrations of caffeine (RCC). Ca^{++} transient in RCC was measured with aequorin method. In RCC, 3 phases of light signals were observed and tension started to rise at the beginning of the second phase. The third phase was triggered after tension reached maximum level. At the first and second phase, oscillatory Ca^{++} transients (about 1.5 Hz) accompanied by small oscillatory tensions were detected. $[Ca^{++}]i$ at the first and second phase was about 3 μM. No parallelism between tension development and the first phase was observed. Procaine mainly inhibited the second and third phase of the light and tension. In the presence of low concentrations of caffeine, Ca^{++} might be released from SR in regenerative manner at 3 μM intracellular free Ca^{++} concentration level.

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EFFECT OF PROCAINE TO CA-UPTAKE MECHANISM ON FRAGMENTED SARCOPLASMIC RETICULUM (FSR) MEMBRANE. NISHIJIMA, H. and SAKAI, T. Dept. of Physiol. The Jikei Univ. Sch. of Med., Tokyo 105 Japan

Effect of procaine to Ca-uptake mechanism on fragmented sarcoplasmic reticulum. It is known that Ca-release from the SR induced with caffeine is inhibited in the presence of procaine. But the inhibitory action of procaine is not clarified. In order to known the inhibitory action in the SR, the present experment was carried out by means of the millipore method. The Ca-uptake in initialphase by procaine was increased, but the maximum volumes of Ca-uptake was same as control FSR. The bound Ca on FSR in the absence of ATP increased by procaine. In the results of this experiment, we searched to the common condition which both release of uptaked Ca and bound Ca were caused by the addition of caffeine or pCa change in reaction solution. The release of uptaked Ca was caused with the condition which the bound Ca on the FSR treated with procaine was released over the level of bound Ca of control FSR. From these results, we considered that the Ca-release from FSR was closely connected with the action of bound Ca in FSR.

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ARSENAZO III SIGNALS IN FROG SKELETAL MUSCLE: EFFECTS OF NITRATE AND ZINC. OCHI, K., MATSUMURA, M. AND TOYOTA, H. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki 701-01

Intracellular Ca^{2+} transients following the action potentials were recorded from arsenazo III-loaded frog twitch muscle fibers. Changes in transparency at the wavelengths of 534 nm and 604 nm were detected by a pair of the photomultipliers, whose output current was differentially amplified and recorded through a cutoff filter of 560 Hz. Usually, arsenazo III signals in 8-32 twitches were averaged. Since the movements during contraction distorted seriously the optical signals, the muscle was obliged to be highly stretched to exclude the artefact. In normal Ringer solution at 20-24°C, the arsenazo III signals started to rise after 3 msec latency and reached the maximum after another 5 msec, and then decayed in nearly exponential manner with a half decay time of 13 msec. When 110 mM Cl^- in normal Ringer solution was replaced with equimolar NO_3^-, the falling phase of the signal was slowed down and the half decay time was nearly doubled. On the other hand, the latency, time to the peak and the maximum amplitude appeared to be little affected by NO_3^-. When 25 μM Zn^{2+} was added to the bathing solution, the falling phase was also markedly prolonged. It is suggested that both NO_3^- and Zn^{2+} increased not the rate of but the duration of Ca^{2+} release from the sarcoplasmic reticulum.

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MECHANISM OF DECOUPLING IN SKELETAL MUSCLES. ISOJIMA,C. Dept. of Physiology,St.Marianna University, School of medicine. Miyamae-ku,Kanagawa 213

External Mn^{2+} has been reported to decouple the E-C coupling of skeletal muscles and to block Ca^{2+} influx during depolarization. In order to clarify the relation between the decoupling and the blocking of Ca^{2+} influx, the effect of external Mn^{2+} on the local contraction of single fibres was examined. In Na^{+}-free RINGER's fluid containing 1.8 mM Ca^{2+}, the threshold ratio was 2.22 in 7.6 mM Mn^{2+}, but was only 1.55 in 15.3 mM Mn^{2+}. In Na^{+}- and Ca^{2+}-free RINGER's fluid, the threshold ratio was 1.63 in 7.6 mM Mn^{2+}. These results may be interpreted to indicate the presence of at least two mechanisms of the inhibitory effect of Mn^{2+}. The one is a change of depolarization in T-tubular membrane due to the blocking of Ca^{2+} influx and the other is a still unknown inhibitory effect on some coupling process intervening between the T-tubular depolarization and the lateral sac Ca^{2+} release. This interpretation is considered to support the view put forward by ARMSTRONG, BEZANILLA and HOROWICZ in 1972.

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Ca-TRANSIENTS AND EXCITATION-CONTRACTION COUPLING IN SINGLE SKELETAL MUSCLE FIBERS OF FROG, R. JAPONICA. M. FUJINO. Y. SATO, T. ARIMA. 1. Dept. of Physiol., Natl. Def. Med. Coll., Tokorozawa.

Time course of Ca-transients (Ca-T) during contraction evoked by single electrial shocks was studied in relatively short fibers (rest length, about 1.5 mm), using an optical technique, where the metallochromic dye, arsenazo III, was injected electrophoretically through glass-micropipette. Ca-T was monitored by the absorbance of a wave length, 650 nm. Results : 1. Ca-T appears slightly before the start of latency relaxation. 2. Temporal parameters (ms) of Ca-T, such as latency (L), peak time (T_p), half duration (D_h), and time-constant of falling phase (τ_f) were not so greatly different among conditions given, normal, streched, NaCl-hypertonic, D_2O-immersed : L; 1.8, 1.7, 1.6, 2.0; T_p, 7.1, 9.2, 10.6, 8.5; D_h, 27, 29, 31, 25; τ_f, 25, 25, 25, 21; respectively. Whereas, those for EGTA-injected condition were somewhat reduced than the above : L, 1.3; T_p, 3.5; D_h, 11; τ_f, 12. 3. Ca-T was enhanced by anions (SCN and NO_3) and caffeine (0.5 mM) and inhibited by dantrolene sodium (saturated).

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PHENYlGlYOXAL (PGO) AND SITES FOR EXCITATION-CONTRACTION (E-C) COUPLING IN SKELETAL MUSCLE CELLS OF FROG, R. JAPONICA. M. FUJINO, Y. SATO, T. ARIMA, H.TAKAI. 1. Dept. of Physiol., Natl. Def. Med. Coll., Tokorozawa.

Studies on effects of acrolein derivatives on E-C coupling brought us to the present studies on PGO, which reacts with guanidyl radical. Results: 1. Treatment of single fibers with PGO of several mM for appropriate durations inhibits K-contracture almost without effects on both membrane potential and caffeine-contracture. The grade of inhibition depends on the intensity of treatment (concentration, time) : Thus, if the intensity is less, the inhibition is reversible. The inhibitory effects of PGO are weakened, if K is substituted partially for Na in Ringer. 2. Similar results are obtained also in whole sartorii. 3. Under these inhibiting conditions, a less stainable portion appears in lateral sacs, and mitochondria are deteriorated. These ultrastructural changes are also protected by substitution of K for Na in Ringer. 4. SDS PAGE-analysis done so far showed that MW of proteins combined with (7-^{14}C)PGO fell between 30,000 and 40,000 dalton. Diltiazem was also shown to inhibit E-C coupling.

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RELATIONSHIP BETWEEN INTERNAL MEMBRANES AND TWITCH TENSION UPON EXCITATION OF T-TUBULE IN FROG SKELETAL MUSCLE. OBA, T., YAMAMOTO, M. AND HOTTA, K. Dept. of Physiology, Nagoya City University Medical School, Nagoya 467

Single fibers dissected from X.laevis iliofibularis upon electrical stimulation contracted with a faster rate and a larger $\dot{T}_{max}$ than did the bull-frog semitendinosus, while T_{max} was the same. Calcium sensitivity and $\dot{T}_{max}$ of mechanically skinned fibers of X.laevis resembled that of bull-frog. X.laevis muscle had a small cross-sectional area of T-tubule and the action potential exhibited a small positive-going hump. These results show that X.laevis muscle appears to be a good model for investigation of mechanisms related to calcium release from SR, as elicited by the T-tubular excitation. Reduction of external calcium from 1.8 mM to 18 μM elicited spontaneous contraction in bull-frog even after addition of 3 mM $MgCl_2$, but not in X.laevis. Potentiation of twitch tensionwas observed immediately after exchange the medium to free calcium solution and then tension disappeared. Bull-frog fiber was more sensitive to external calcium reduction than that of X.laevis.

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EFFECTS OF pH AND IONIC STRENGTH TO RADIAL STIFFNESS IN SKINNED MUSCLE FIBERS. UMAZUME,Y. AND ONODERA,S. Dept. of Physiol., Jikei Univ. Sch. of Med., Minato-ku Tokyo 105

Radial stiffness of skinned fibers may reflect various interaction energies between filaments (an electrostatic repulsive energy, the van der Waals atractive energy and hydration energy). Adding PVP K-30 in incubating solution, the width of the fiber (D) was measured under various pH and ionic strength conditions. In relaxing solution, radial stiffness decreased markedly in pH 6 solution and increased slightly in pH 8. Radial stiffness in ionic strength of 0.06, 0.15 and 0.25 M solutions showed no significant deference. In rigor solution, simillar effects of pH were observed.

RADIAL STIFFNESS $N/m^2 \times 10^{-4}$, MEAN(S.D.)

	D/D_0 (D_0: width of the fiber in pH 7 solution)					
	1.00	.96	.92	.88	.84	.80
pH 6	-	.55(.07)	.64(.09)	.76(.11)	.95(.12)	1.26(.16)
pH 7	.62(.06)	.69(.07)	.80(.07)	.98(.07)	1.34(.12)	2.37(.36)
pH 8	.69(.04)	.79(.02)	.92(.07)	1.19(.27)	1.90(.81)	3.14(.72)

Effect of pH to radial stiffness suggest that one of the factors of the repulsive force is the charge of the filaments.

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INHIBITORY EFFECTS OF TWITCHES ON THE TETANIC CONTRACTION OF FROG SKELETAL MUSCLE. KAWATA, H. and HATAE, J. Dept. of Physiology, Faculty of Medicine, Fukuoka University, Jonan-ku, Fukuoka 814-01

The effects of twitch trains, which are showing an ascending staircase phenomenon, on the following tetani were investigated on the small bundles excised from bullfrog's toe muscle. Tetani of 50 Hz for 1 sec were induced every 5 min as the basal condition. When twitch trains of various frequencies (above 1 Hz) and of numbers (below 250) were interposed between two successive tetani, three different effects were observed during the following tetani; a rapidly decaying potentiation (P_1), a slowly decaying potentiation (P_2) and an inhibition (I) which was most dominant. Increasing the twitch frequency resulted in an enhancement of all of these after-effects. By lowering the bath temperature (4°C) the potentiating effect (P_2) was masked by marked inhibition (I), leaving P_2 remained unchanged. Elevating the temperature (28°C) resulted in a reverse effect. Caffeine (0.5 mM) mimicked the effect of low temperature. The results suggested the possibility that calcium turnover process at the sarcoplasmic reticulum may play some role in the after-effects, especially in the inhibition.

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DEVELOPMENTAL CHANGES IN MUSCULAR POTENTIALS INDUCED BY PROLONGED REPETITIVE STIMULATION IN GASTROCNEMIUS MUSCLES OF DYSTROPHIC AND NORMAL MICE.
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With urethane-anesthetized dystrophic and littermate normal mice of various ages (from 14 days of age up to adult), muscular potentials were evoked in the gastrocnemius muscle by repetitive sciatic stimulation. When the frequency of stimulation increased from 0.5 to 5 Hz and maintained, muscular potentials were reduced in amplitude to a considerable extent in normal mice, and the amount of reduction was greater in older than in younger normal mice. Dystrophic mice fell into two classes, showing a slight decrease or increase in muscular potentials due to 5 Hz-stimulation, and the magnitude of these changes was almost constant in dystrophic mice regardless of age. A characteristic contrast between postnatal development of dystrophic and normal mice was thus revealed in the present study.

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MATURATIONAL DEFECT OF MUSCLES IN DWARF AND DYSTROPHIC MICE.
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For the pathogenesis of murine muscular dystrophy, we have proposed a bone-muscle imbalance hypothesis consisting of several steps, with the primary stage (arrest of muscle growth) and the subsequent ones dependent upon bone growth. This hypothesis was in part evidenced by the fact that muscular dystrophy was almost latent in genotypically dystrophic-dwarf mice. In the present work, muscles (forelimb triceps and hindlimb rectus) of dwarf (DW/J dw/dw) and dystrophic (C57BL/6J dy/dy) mice were compared histologically. In dwarf muscles, three types of fibers (dark, intermediate and pale; NADH-TR staining) were distributed almost normally, though they were much thinner than their corresponding normal fibers. In dystrophic muscles, a diffused distribution of dark fibers with small diameter was prominent and central nuclei (HE staining) were observed in many fibers regardless of the fiber type and diameter.

It was concluded that a defective maturation, not a progressive degeneration followed by a partial regeneration, of muscle fibers might be characteristic of murine muscular dystrophy.

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SARCOMERE LENGTH AND FORCE CHANGES IN RESPONSE TO SINUSOIDAL LENGTH CHANGES IN SINGLE FROG SKELETAL MUSCLE FIBERS.
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To obtain information about the mechanical properties of tetanized frog skeletal muscle fibers, sinusoidal length changes (amplitude, 0.3% of L_o; frequency, 20-3 kHz) were applied to single muscle fibers during tetanus, and the resulting sarcomere length and force changes were recorded. Data were only obtained from fibers which showed sinusoidal sarcomere length change signals in response to the applied sinusoidal fiber length changes. The sarcomere length changes measured at the middle of the fiber decreased with increasing frequency of the fiber length changes, while the force changes measured at the fixed fiber end increased. The phase differences between the fiber and the sarcomere length changes were nagative, decreasing slightly with increasing frequency. The phase differences between the fiber length and the force changes were positive, decreasing with increasing frequency. These results are explained by a viscoelastic multi-sarcomere model with a series elastic component in each sarcomere.

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ON THE AMINOPYRIDINE RAPID COOLING CONTRACTION IN SLOW AND FAST MUSCLE.
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The combined effects of 4-aminopyridine (4AP) and cooling bring contraction of the muscle (AP-RCC) and activation of the endplate. AP-RCC and endplate activation were investigated in three different muscle fibers of bullfrog; toe muscle, slow (S muscle) and fast muscle (F muscle) bundles dissected from the iliofibular muscle. 4AP was more effective to induce AP-RCC in toe and S muscle than in F muscle. The effects of d-TC or Mn to inhibit AP-RCC on these muscle were indistinguishable. During AP-RCC, there take place endplate potential (cooling epp) or small motor nerve junctional potential (cooling sjp) spontaneously and repetitively. The amplitude of AP-RCC and the frequency of cooling epp or cooling sjp diminished by increase of external Ca concentration being less effective on cooling sjp. It was concluded that the nerve terminal to F muscle was less sensitive to 4AP, and that the nerve terminal to S muscle was less sensitive to Ca concentration.

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STUDIES ON THE LONGITUDINAL STABILITY IN FROG SKELETAL MUSCLE.
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In order to study the longitudinal stability in active muscle, a pair of frog sartorii were attached to either end of a lever pivoted at the middle. By changing the point of attachment of one of the muscles, we could produce graded inequalities between the two muscles. The lever was initially fixed in position, so that the muscles were first tetanized isometrically. When the isometric forces reached a plateau, the lever was released to move depending on the degree of inequalities caused by the lever arm ratio of 2-10. The force-velocity relation of the advantaged muscle, which shortened by stretching the disadvantaged one, was identical with that obtained from the conventional isotonic releases under constant loads. The force-velocity relation of the disadvantaged muscle, which was lengthened by the advantaged one, was found to be different from that obtained from the conventional experiments; the velocity of lengthening of the disadvantaged muscle was very slow, while the force exerted on the disadvantaged muscle exceeded 2 P_o. This indicates that the load-bearing ability of active muscle is heavily dependent on the past history.

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MODE OF SARCOMERE SHORTENING IN STRIATED MUSCLE.
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Stepwise sarcomere shortening has been recorded in intact striated muscle fibers with several optical methods, though it remains to be determined if the stepwise sarcomere shortening is genuine or due to optical artifacts. To obtain more accurate information about the steps and pauses, we studied the shortening behavior of very small preparations (bundles of myofibrils made from glycerinated frog skeletal and intact, single frog cardiac muscle fibers) with phase-locked loop device and high speed videocamera (5 msec time resolution), and traced the shortening behavior of the same sarcomere groups. These preparations were 5-15 μm wide and 100-200 μm long. The myofibril preparations were activated by raising free [Ca], while the cardiac fibers were by electrical pulses. Both types of the preparations showed pauses during shortening. Especially the cardiac fibers regularly exhibited a pause after a small amount of initial rapid shortening, the duration of the pause depended on the shortening velocity which was changed by changing external [Ca]. The results imply that the shortening of the sarcomere may not be smooth but stepwise.

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THE RELATION BETWEEN SHORTENING HEAT AND SARCOMERE LENGTH IN FIBER BUNDLES OF FROG SKELETAL MUSCLE.

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The relation between the shortening heat and the sarcomere length was studied using fibre bundles from frog semitendinosus muscles as well as using whole muscles. The shortening heat was estimated as the excess heat produced during 0.4 sec after the start of rapid isovelocity releases in a 3 sec tetanus at 0°C.

The apparent shortening heat in whole muscles became negative at very long sarcomere length (> 3 μm). The unstimulated whole muscles showed a large thermoelastic absorption of heat when released at long sarcomere length (> 2.5 μm). Hence the apparent shortening heat was corrected for the thermoelastic heat absorption. The resulting shortening heat decreased linearly with sarcomere length. The thermoelastic heat absorption at long sarcomere length was much reduced in fibre bundles, showing that the structure that causes the thermoelasticity exists mainly outside muscle cells. After the correction the shortening heat in fibre bundles decreased linearly with sarcomere length, which is quite similar to that in whole muscles. The results are interpreted by that the shortening heat is closely associated with the crossbridges and that the origin of the shortening heat may be the shift in the population of crossbridge states during rapid shortening.

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STRETCH-INDUCED TENSION DEVELOPMENT IN GUINEA-PIG TAENIA COLI. TSUCHIYA, T., KIMURA, H., TORIDE, M. and SUGI, H. Dept. of Physiology, School of Medicine, Teikyo University, Itabashiku, Tokyo 173

Quick stretch applied to an isolated strip of guinea-pig taenia coli elicited tension development after a short delay. The height of delayed tension was linearly related to the increased amount of muscle length within 40 % of the initial length. The responses to stretch were not affected by tetrodotoxin and atropine. In the presence of Mn^{2+} , D600 and verapamil, however, the responses were completely abolished and the removal of Ca^{2+} from the bathing solution abolished the responses as well. At the higher external Ca^{2+} concentration, the responses were reduced with the increase of the concentration (-20 mM). On the depolarized muscle by high potassium concentration (100 mM), no responses were observed, though the low concentration (10 mM) augmented them. All the responses observed were accompanied by spike discharges, measured by sucrose-gap technique. Above results suggest that the discharges of Ca-spikes, hence the entry of Ca^{2+}into the cells, are essential to the delayed tension development after stretch.

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ISOMETRIC CONTRACTION OF SINGLE ISOLATED SMOOTH MUSCLE CELLS. Kosaka, I. and Yabu, H. Dept. of Physiology, Sapporo Medical College, Sapporo 060 Japan

Single smooth muscle cells were isolated, by the treatment with collagenase (Sigma, Type I) and dispase (Godo Shusei, Type II), from stomach circular muscle of the newt, Cynops pyrrhogaster. Muscle tissue, from which mucosa and serosa have been removed, was incubated in Ca-free, Mg-free Ringer solution at room temperature. After 15 min, the tissue was treated with Ca-free, Mg-free Ringer solution including 0.05 - 0.1% collagenase and 0.2 - 0.6% dispase at 30 - 35°C for 30 - 45 min. The tissue was then incubated again in Ca-free, Mg-free Ringer solution at room temperature. At this time single cells of around 800 um in length started to be dispersed gradually. About 100 um of each end of the isolated cell was sucked into the tip of the pair of glass micro-capillarys, one of which has a compliance of 48 um/mg and another less than 1 um/mg. Isometric contraction was recorded by photoelectrically detecting the displacement of the tip of micro-capillary with a larger compliance. At about 0.5 L_o (L_o means the relaxed length after dispersion without any stimulation), a cell developed the tension of about 130 ug (360 g/cm^2) with a lag of 500 msec and a time to peak of about 8 sec by 4 X 10^{-7}A and 100 msec rectangular current stimulation. Another cell developed the tension of about 250 ug (720 g/cm^2) with 750 msec lag and 8 sec time to peak at about 1.0 L_o. The tension record was carried out at room temperature (22°C).

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COMPUTER SIMULATION OF ISOMETRIC TENSION CURVE IN UTERINE SMOOTH MUSCLE. KATO, S., OGASAWARA, T., AND OSA, T. Department of Physiology, Yamaguchi University, School of Medicine, Ube 755.

A kinetic model was proposed to simulate an isometric tension curve in uterine smooth muscle on the basis of the myosin phosphorylation hypothesis. The Ca^{2+}-calmodulin-dependent activation of myosin light chain kinase and the phosphorylation-dephosphorylation reaction of myosin were mathematically treated. Solving the kinetic equations at a steady state, we could calculate the relationship between the Ca^{2+} concentration and the myosin phosphorylation. Assuming that two-head-phosphorylated myosin has an actin-activated Mg^{2+}-ATPase activity and this corresponds to an active state, we computed the time courses of the myosin phosphorylation and the active state for various Ca^{2+} transients. The time course of the active state was converted into isometric tension by use of Sandow's model composed of a contractile element and a series elastic component. The proposed model could simulate not only the isometric tension curves for any given Ca^{2+} transient but also many experimental results on smooth muscle contraction at issue.

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EFFECT OF SODIUM DEPLETION ON THE ELECTRICAL AND MECHANICAL ACTIVITIES IN THE SMOOTH MUSCLE OF THE RAT PORTAL VEIN. YAMAMOTO, Y. and HOTTA, K. Dept. Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467.

Electrical and mechanical activities of the smooth muscle of the rat portal vein were investigated with a microelectrode and a isometric force-transducer. When the external Na was replaced with choline or Tris, the membrane depolarized to about -30mV and the muscle tone increased gradually (Na-free contracture). However, using Li as Na substitute, Na-free contracture did not occur in spite of the membrane depolarization. Since pre-treatment for neither loading nor depleting the intracellular Na affected the Na-free contracture, contribution of the Na-Ca exchange mechanism to this contracture seems to be little. The Na-free contracture did not appear in a Ca-free solution with 0.5mM-EGTA, but addition of 2mM-Mn to this solution restored the contracture. The Na-free contracture was temperature-dependent and it did not occur at the temperature below 15°C. These results indicate that the intracellular Na may play an important role to control the membrane permeability for Ca and that the depletion of this Na may allow the external Ca or Mn to enter the cell.

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EFFECTS OF THEOPHYLLINE ON THE CONTRACTURE PRODUCED BY Na REMOVAL IN THE STOMACH MUSCLE OF GUINEA-PIG. ASHOORI,F., TOKUNO,H., AND TOMITA,T. Dept. of Physiol., Sch. of Med., Nagoya Univ., Nagoya

Na removal produced depolarization and contracture in the circular muscle of guinea-pig stomach. The contracture was potentiated by indomethacin, suggesting that Na removal releases inhibitory prostaglandins. Isobutyl methylxanthine, theophylline and caffeine suppressed both depolarization and contracture due to Na removal, the potency being in this order. Verapamil strongly inhibits the contracture but not the depolarization when given after their full development, although pretreatment with verapamil reduced the early phase of depolarization. Dibutyryl cyclic AMP (3×10^{-4}M) markedly suppressed the contracture, while it had no significant effect on the depolarization. This may indicate that the suppression of the contracture by xanthine derivatives is partly due to cyclic AMP through inhibition of phosphodiesterase, but that the prevention of depolarization is not related to blockade of the Ca conductance and also to an increase in cyclic AMP.

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CONTRIBUTION OF GLYCOLYSIS IN THE SPONTANEOUS ACTIVITY IN THE CIRCULAR MUSCLE OF GUINEA-PIG STOMACH. KOBAYASHI,T., KASAHARA,H., ASHOORI,F., TOKUNO,H., TAKAI,A. AND TOMITA,T. Dept. of Physiol., Sch. of Med., Nagoya Univ., Nagoya

The circular muscle of the guinea-pig stomach produces regular (3-5/min) spontaneous activity. The frequency was slowly reduced in the majority of preparations in glucose-free solution. The frequency was restored by glucose, but not by pyruvate or beta-hydroxybutyrate. In the absence of glucose, the activity quickly stopped by CN (1-2 mM) or anoxia, but in the presence of glucose, some activity remained in most of preparations. Under the latter condition, pyruvate application produced nearly normal activity and increased lactate production 20 times, suggesting that ATP supply can be maintained only with glycolysis. 2-Deoxyglucose lowered the frequency without reducing O_2 consumption. Monoiodoacetate blocked the activity which could not be produced by pyruvate or beta-hydroxybutyrate, although O_2 consumption and high energy phosphate compounds were not significantly different from the control. From these results it was suggested that glycolysis is indispensable for the generation of the rhythmic activity.

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CONTRACTION AND GLUCOSE METABOLISM IN MYOMETRIUM - THEIR CHANGES WITH PREGNANCY. MASAHASHI,T. AND TOMITA,T.* Dept. of Obstet. Gynecol. and Dept. of Physiol.*, Sch. of Med., Nagoya Univ., Nagoya

In the rat myometrium the reduction of external Na concentration produced a sustained tonic contracture (0 Na contracture). In the non-pregnant myometrium removal of external glucose or application of 5 mM 2-deoxyglucose, which is supposed to inhibit the glycolysis, slightly suppressed the 0 Na contracture, whereas 1 mM CN, which inhibits the electron transfer in mitochondria, markedly suppressed the 0 Na contracture. In the pregnant myometrium, on the other hand, inhibition of glycolysis strongly suppressed the 0 Na contracture and the suppression by CN became weaker when the 0 Na contracture was repeated. Simultaneous application of both 2-deoxyglucose and CN almost completely abolished the 0 Na contracture in both the non-pregnant and pregnant myometrium. But even in such a condition pyruvate (11.8 mM) produced a significant recovery of the tension development in the non-pregnant myometrium. It is speculated that during pregnancy the role of glycolysis becomes important in the maintenance of the contraction.

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ROLES OF Ca AND Na IN VANADATE-INDUCED CONTRACTURE OF SMOOTH MUSCLES. SUNANO, S. Dept. of Physiol., Sapporo Medical College, Chuo-ku, Sapporo 060

Effects of Ca and Na on vanadate-induced contracture of smooth muscles of guinea-pig vas deferens, ureter and portal vein were studied. Vanadate at the concentrations higher than 10^{-4} M induced contracture both in polarized and depolarized preparations. Removal of extracellular Ca caused a reduction of the developed tension by about 50 percent. Remaining tension was observed even after soaking the preparations in Ca-free solution for 120 min, and was insensitive to Ca antagonists. Ca-induced contractures of depolarized preparations were potentiated in the presence of vanadate and lower concentrations of Ca could induce the contracture. Vanadate-induced contractures were potentiated in the presence of Na and diminished in the absence of Na. Similar results were obtained in Ca-induced contracture of depolarized preparations observed in the presence of vanadate. The addition of Na to Na-depleted preparations in the presence of vanadate caused the tension development. Ouabain showed no effect on the vanadate-induced contracture and on the action of Ca and Na. It was demonstrated that vanadate initiated extracellular Ca-dependent and -independent contractures in smooth muscles. These contractures were influenced by extracellular Na in Na,K-ATPase-independent manner.

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EFFECTS OF VERAPAMIL ON THE GUINEA PIG TRACHEAL SMOOTH MUSCLE. KAWANISHI, M., BABA, K* and TOMITA, T** Dept. of Anesthesiol., 2nd Dept. of Int. Med* and Dept. of Physiol**, Sch. of Med., Nagoya Univ., Nagoya

In the guinea pig tracheal smooth muscle, the tonic contraction is Ca concentration-dependent in 5.9 mM K (Krebs) and in 40 mM K solutions. Complete removal of external Ca produces complete relaxation, suggesting that Ca influx regulates the contraction. But the mechanism of Ca influx seems to be different when depolarized by excess K. PGs have been reported to play a main role in the maintenance of tone in this preparation. In Krebs solution the tension is also abolished completely by indomethacin, a PGs synthesis inhibitor, at 5×10^{-6} M. The effects of PGs and 40 mM K were compared, therefore, in the presence of indomethacin and of verapamil, a Ca-channel blocker. The contraction produced by PGs (PG $F_{2\alpha}$ and PG E2) was not affected, while that produced by 40 mM K was markedly suppressed by verapamil (10^{-5} M). It is speculated that at least two routes, a verapamil-sensitive (probably voltage-dependent Ca-channel) and a verapamil-insensitive (PGs mediated) route, are responsible for the Ca influx in the tracheal smooth muscle.

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EFFECTS OF DIVALENT CATIONS ON THE CONTRACTION-RELAXATION CYCLE IN GASTROINTESTINAL SMOOTH MUSCLE. SAKAI, Y., OOUCHI, M., ISOBE, A., ICHIKAWA, S. Dept. of Physiol. Showa Univ. School of Med. Shinagawa-ku, Tokyo 142

The effects of divalent cations on Ca^{2+} movement by plasma membrane enriched fractions (PM) isolated from canine stomach was studied to determine the relations of divalent cation interactions with the contraction-relaxation cycle in intact smooth muscle. Ca channel could pass the divalent ions, Ba^{2+} and Sr^{2+}, since Ca^{2+} entry blocking agents, verapamil and Co^{2+}, inhibited contraction induced by these cations. After Ca^{2+} depletion, Sr^{2+} mimicked the role of Ca^{2+} less efficiently as an intracellular activator of contraction. Muscle tone was not decreased after washout of Sr^{2+}. Sr^{2+} inhibited Ca^{2+} uptake by PM in both the presence and absence of ATP. Release of Ca^{2+} from PM vesicles was enhanced by Sr^{2+}. Sr^{2+} could replace Ca^{2+} in its movement since this cation competes with Ca^{2+} for binding sites. Results suggest that Sr^{2+} could substitute for Ca^{2+} and replace it in a Ca^{2+} pool in the cell. Therefore, intracellular free Ca^{2+} concentration might be increased by Sr^{2+} to maintain basal tone.

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MECHANISM OF PRECIPITATION-REACTION BETWEEN PYROANTIMONATE AND INTRACELLULAR CATIONS IN THE SMOOTH MUSCLE. SUZUKI, S., KAMIYAMA, A. AND SUGI, H. Dept. of Physiol., Sch. of Medicine, Teikyo University, Itabashi-ku, Tokyo 173

X-ray microanalysis of pyroantimonate precipitate was carried out to ascertain whether pyroantimonate serves as a valid measure of Ca localization in smooth muscle cells. Cryo-sections of the anterior byssal retractor muscle (ABRM) of Mytilus edulis were prepared after fixation with the pyroantimonate-osmium solution and after dehydration with ethanol. In these cryo-sections and the ordinary Epon-embedded sections, the pyroantimonate precipitate was examined by means of electron probe X-ray microanalysis. The concentration of Ca (relative to Sb) in the precipitate was nearly constant irrespective of the stages at which the sections were prepared. While the concentration of K, Na and Mg changed remarkably depending on the stages examined. These results indicate that the intracellular pyroantimonate precipitate formation is primarily determined by Ca ions, and that the pyroantimonate method is valuable for detecting the intracellular Ca localization. To emphasize this evaluation for the pyroantimonate method, by means of X-ray microanalysis, Ca was directly detected from a cryo-section which was obtained from the ABRM preparation without chamical fixation by glutaraldehyde and/or osmium tetroxide, and the result was also discussed.

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A POSSIBILITY OF INTRACELLULAR Ca RELEASE IN NORADRENALINE(NA) RESPONSE OF THE RAT VAS DEFERENS IN Ca-FREE SOLUTION. TAKAI,A., ASHOORI,F. AND TOMITA,T. Dept. of Physiol., Sch. of Med., Nagoya Univ., Nagoya

The epididymal part of the rat vas deferens could be repeatedly contracted by noradrenaline through alpha 1 receptor activation in the Ca-free Krebs solution containing 0.5 mM EGTA. A calmodulin antagonist, W-7 (3x 10^{-4}M), strongly inhibited the contraction, and the Ca content of the tissue incubated in Ca-free solution slowly decreased with a half time of 2.8 hrs. Therefore the contraction seems to be due to Ca released from intracellular sites, such as sarcoplasmic reticulum or mitochondria. The relaxation phase of the NA response was inhibited by a prolonged exposure (more than 1 hr) to glucose-free solution. Monoiodoacetate (10^{-4}M) or carbonyl cyanide m-chlorophenyl hydrazone, an uncoupler, also blocked the relaxation phase. These metabolic inhibitors reduced the Ca content as well as the amount of the high energy phosphate compounds in the tissue. Therefore, the recovery of the NA response is probably linked with a Ca-pump or Ca-reuptake through an ATP-dependent process. However, the sites of intracellular Ca store cannot be determined from these experiments.

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THE ENHANCEMENT OF ELECTRICALLY EVOKED CONTRACTION BY MEMBRANE DEPOLARIZATION IN GUINEA-PIG VAS DEFERENS. WAKUI,M. Dept. of Applied Physiology, Tohoku University School of Medicine, Seiryocho,Sendai 980

Noradrenaline(NA) increased the magnitude of contraction evoked by depolarizing current stimulation to the smooth muscle membrane of guinea-pig vas deferens with a few mV resting membrane depolarization and increased electrical membrane activity under a double sucrose-gap method. In low Na solution NA had no effect on the evoked contraction, but with subcritical membrane depolarization by current application resulted bigger contraction. Meanwhile depolarization by current application in 10 mM TEA included solution enhanced the evoked contraction even with nearly the same electrical membrane activity. On the other hand, the contraction by depolarizing current stimulation was observed even in Ca-free solution, and in this environment subcritical membrane depolarization prior to current stimulation had no effect on the evoked contraction. It is concluded that subcritical membrane depolarization may increase the amount of internally stored Ca probably through Na-Ca exchange system.

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MODULATING ACTION OF EXOGENOUS ATP ON MEMBRANE POTENTIAL, CURRENT AND CONTRACTILE TENSION IN THE SMOOTH MUSCLE OF GUINEA-PIG VAS DEFERENS. INOMATA, H. AND WAKUI, M. Dept. of Applied Physiology, Tohoku University Medical School, Sendai 980

The effects of ATP on membrane potential, current and contractile tension in the smooth muscle of guinea-pig vas deferens were studied under current and voltage clamped conditions. A Krebs solution in which 50% Na ion was replaced by TEA ion was used. An addition of ATP (1×10^{-5} g/ml) produced immediate positive inotropic effects in current clamped condition; the amplitude of action potential was enhanced, the maximum dV/dt grown rapid and the repolarizing phase shortened without any detectable change in the resting membrane potential while dV/dt dependent tension was developed. Such a positive inotropic response was kept constant in the presence of ATP.

Under voltage clamped condition, the addition of ATP increased the maximum Ca ionic current (I_{Ca}) and shifted significantly its reversal potential (E_a) towards positive voltages with increasing the chord conductance for Ca ion ($\bar{g}_{Ca}$), while the outward K ionic current was enhanced by increasing the chord conductance for K ion.

From the above results, it can be suggested that ATP at lower concentration might cause the positive inotropic effects mainly by activating Ca ion influx through voltage sensitive Ca ionic channels rather than by opening the receptor Ca ionic channels.

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STUDIES ABOUT THE RELATIONSHIP BETWEEN SLEEP AND ARRHYTHMIAS. SATO, T., OTSUKA, K., SAITO, H., YOSHIMATSU, K., KABA, H., SETO, K., NOJIMA, K.*, SATO, Y.**, ICHIMARU, Y.** and YANAGA, T.** Dept. of Physiology, Kochi Medical School, Kohasu, Oko-cho, Nankoku-city, 781-51, *Common Experimental Center, Kochi Medical School, **Dept. of Bioclimatology and Medicine, Research Institute of Bioregulation, Kyushu University

The effects of bilateral suprachiasmatic nucleus (SCN) lesions on the circadian rhythm of heart beats and cardiac arrhythmias were studied in 5 male Wister rats under a 14/10 light-dark schedule. Biploar standard leads (I, II and III) were used for analyses of arrhythmias and cortical and hippocampal EEGs were used to monitor the sleep state. Bradyarrhythmias such as sinus arrest, type I and II second degree atrioventricular block were observed both in 5 control rats and in 5 SCN-lesioned rats. SCN lesions were confirmed by the elimination of the circadian rhythm of sleep and locomotor activity and by postmortem histological examination. In control rats the circadian rhythm of heart beats and arrhythmia episodes was observed. In SCN-lesioned rats these circadian rhythms were completely eliminated, but arrhythmogenic properties of paradoxical sleep observed in control rats were maintained.

These results suggest that the elimination of the circadian rhythm of heart beats and arrhythmia episodes may be related to the elimination of sleep-wakefulness rhythm by the SCN lesions.

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STUDIES OF THE CENTRAL NERVOUS CONTROL OF THE ATRIOVENTRICULAR CONDUCTION. NOJIMA, K., OTSUKA, K.*, SAITO, H.*, KABA, H.*, YOSHIMATSU, K.*, SETO, K.*, ICHIMARU, Y.**, SATO, Y.** and YANAGA, T.** Common Experimental Center and *Dept. of Physiology, Kochi Medical School, Kohasu, Oko-cho, Nankoku-city, 781-51, **Dept. of Bioclimatology and Medicine, Research Institute of Bioregulation, Kyushu University

The effect of the ventromedial hypothalamus (VMH) on the atrioventricular (A-V) conduction disturbances induced by ouabain was investigated in male Wister rats. ECG records were obtained from the bipolar standard lead II and from the X, Y and Z vectorial leads. The His bundle potential from body surface was recorded by 2,000 beat signal averaging of these scalar ECGs. High pass filter settings of 80 Hz appeared optimal for rejection of the low frequency components. The bilateral VMH were lesioned electrically (D.C. 3mA, 60 sec) using a monopolar platinum electrode of 0.5 mm diameter, and the following doses of ouabain were given intraperitoneally at 30 min intervals; 1, 3, 5, 10, 15, 20, 25, 30, 35 and 40 mg/kg. P-Q, A-H and H-V intervals were measured before and after one hour after the VMH lesions in intact, sham-operated and operated rats. These intervals showed no significant changes. However, ouabain-induced A-V conduction disturbances were enhanced in the VMH-lesioned rats compared to the intact and sham-operated rats. These results suggest that the VMH may participate to play roles in the regulation of the A-V conduction disturbances induced by ouabain.

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A STUDY OF ELECTROCARDIOGRAPHIC T WAVE ON THE BASIS OF ACTION POTENTIAL DURATION AND Na,K-ATPase ACTIVITY IN THE VENTRICLE OF SIMIAN (MACACA FUSCATA) HEARTS. Imanishi, S., Aomine, M., Arita, M. and Kiyosue, T. Dept. of Physiology, Faculty of Medicine, Medical College of Oita, Oita 879-56

The polarity of T wave was analyzed from the standard 13 leads electrocardiogram (ECG) taken from simian under secobarbital anesthesia. Direction of mean T vector of ECG suggests that the sequence of repolarization in ventricle may occur from epicardium (EPI) to endocardium (END) and/or from apex to base of the heart. As expected, action potential duration (APD) of END was significantly longer than that of EPI. Similarly, APD of base was longer than apex. On the other hand, Na,K-ATPase activity was significantly higher in EPI than END, while there is no significant difference between base and apex. Ouabain produced more shortening of APD in END while the shortenings caused by changes in external K concentration from 0 to 5.4 mM and by increasing driving frequency were also more pronounced in END. Thus Na,K-ATPase activity seems not to be a main determinant to produce the differences in APD between END and EPI, or base and apex, arguing against hypothesis proposed by I. Cohen et al (Nature 262:657, 1976). They claimed that longer APD was caused by lower K^+ concentrations in extracellular space, i.e., low K^+ conductance, which would be secondary to the higher activity of membrane ATPase.

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DIFFERENCE OF DNP EFFECTS ON THE ACTION POTENTIALS OF CARDIAC MUSCLES EXCISED FROM VARIOUS PARTS OF THE CANINE VENTRICLE. KAMIYAMA, A. and SHIBAYAMA, R. Dept. of Physiology, School of Medicine Teikyo University, Itabashi-ku, Tokyo 173

Changes in parameters of the action potential by the application of 10^{-4} M DNP were studied with the microelectrode technique in Purkinje fibers, subendocardial ventricular muscles, and right- and left-subepicardial muscles, respectively. Maximum rate of rise, amplitude and 50% duration were measured as main parameters, and sometimes the measurement of mechanical responses were added. In all kinds of muscles described above, all parameters diminished within 5 minutes after the application of DNP. The order of rate of diminishing was as follows: Purkinje fiber > subendocardial ventricular muscle > left subepicardial muscle > right subepicardial muscle. After that, the Purkinje fiber and the subendocardial ventricular muscle trended to recover to control even in the medium containing DNP, whereas the subepicardial muscle never recovered in the DNP containing medium. However, recovering in the Purkinje fiber and the endocardial ventricular muscle was abolished by the pretreat of 10^{-6} M ouabain. Preaddition of 50 mM glucose prolonged slightly the time course of diminishing in the values of parameters. From the results mentioned above, it was concluded that the cells in the various portions of the canine ventricle respond in the same manner, except the spontaneous recovery.

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EFFECTS OF REMOVAL OF AMMONIUM ION ON ELECTRICAL AND MECHANICAL ACTIVITIES OF MAMMALIAN MYOCARDIUM. IMANAGA, I., KAMEI, R., KUROIWA, M. Dept. of Physiology, Sch. of Medicine, Fukuoka Univ., 45-1-7, Nanakuma, Jonan-ku, Fukuoka 814-01, Japan

When NH_4Cl (41mM), for which NaCl was substituted, was removed with HCO_3^--CO_2 buffer solution (pH:7.4) after it was perfused for 10 minutes, membrane potential (MP), action potential duration (APD) and twitch tension of isolated canine ventricular papillary muscle were recovered to the control value within a few minutes. However, the removal of NH_4Cl with Tris buffer solution (pH:7.4) depolarized MP about 10 mV, prolonged APD by about 150% of the control and reduced tension. The prolongation of APD was inhibited by higher $[Ca^{++}]_o$. A change from Tris to HCO_3^--CO_2 buffer solution during the removal of NH_4Cl, the depolarized MP, the prolonged APD and the reduced tension were restored to the control value in a few minutes, but in the presence of DNP (10^{-5}M), the restoration was not observed. Removal of $(NH_4)_2SO_4$ (20mM), for which Na_2SO_4 was substituted, with HCO_3^--CO_2 buffer solution remained a depolarization of MP, a prolongation of APD and a reduction of tension for over 30 minutes. Removal of NH_4NO_3 (41mM), for which $NaNO_3$ was substituted, with HCO_3^--CO_2 buffer solution restored MP, APD and tension to the control value very quickly. These results suggest that intracellular acidosis produces a depolarization of MP, a prolongation of APD and a reduction of contractile force, and that active Cl^--HCO_3^- exchange plays a role in acid extrusion.

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EFFECT OF ISOPROTERENOL ON THE SLOW RISING PHASE AND VERY EARLY PLATEAU PHASE, AND THE DURATION OF THE ACTION POTENTIAL OF CANINE VENTRICULAR MUSCLE. Hotta, M., Shibasaki, T., Hirakawa, S. 2nd Department of Internal Medicine, Gifu University School of Medicine, Tsukasamachi, Gifu

We have been working on the action potential(AP) of canine right ventricular papillary muscle, with a partition chamber. The level of AP at the end of 5msec after the beginning of the fast upstroke was defined as PP_5. Maximum dV/dt($\dot{V}$max) of the fast upstroke was also recorded. We constructed PP_5-$\dot{V}$max-plots(Y-X-plots), and also, APD_{50}-RP-plots(Y-X-plots). The APD_{50} meant 50% duration of the AP, and the RP meant "resting" potential.

Isoproterenol(ISO) caused PP_5-$\dot{V}$max-plots to shift upwards(depolarization), with Km value of 5.0×10^{-7}g/ml. ISO also shortend the APD_{50}. ISO caused APD_{50}-RP-plots to shift downwards(shortening) with Km value of 5.8×10^{-8}. These results support, partially, the validity of the older investigation from this laboratory concerning PP_5 and APD_{50}, performed by use of the propagated AP.

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RELATIONSHIP BETWEEN ATRIAL PRESSURE AND MORPHOLOGY OF BULLFROG EVERTED SINUS-ATRIUM PREPARATION. HORIUCHI,E., and HAYASHI,H. Dept. of Physiology, Saitama Medical School, Moroyama, Iruma-gun, Saitama 350-04

Everted sinus-atrium preparations of bullfrogs were prefixed in 2.5% glutaraldehyde under various atrial pressures in the range of 0 to 12 cmH_2O. Atrial muscle fibers were observed by electron microscopy, and the lengths of sarcomeres, A-bands and I-bands were measured. Sarcomere lengths under 0, 1, 3, 6, and 12 cmH_2O pressures were 2.16, 2.48, 2.58, 2.70 and 2.97 μm, respectively. The length of A-band was not much different within the range of 0 to 12 cmH_2O(approximately 1.36 μm), whereas that of I-band under 0 cmH_2O(0.84 μm) was almost half compared with that under 12 cmH_2O (1.62 μm). Mean sarcomere length of left atrial muscles(2.57 μm) was greater than that of right atrial muscles(2.33 μm) under an pressure of 1 cmH_2O, while the former (2.88 μm) was significantly less than the latter(3.26 μm) under 12 cmH_2O. The relationship between the isometric tension and sarcomere length of the bullfrog atrial muscle fibers was compared with that of frog skeletal muscle. The falling limb of the curve on the former was approximately coincided with that of the latter, however, the ascending limb of the former was greately shifted to the right.

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EFFECTS OF ATP ON THE ISOMETRIC TENSION AND THE TRANSIENT TENSION RESPONSE TO STEP LENGTH STRETCH. SATO, C., SAEKI, Y. AND YANAGISAWA, K. Dept. of Physiol., Tsurumi Univ. Sch. Dent. Med., Yokohama

At a muscle length Lo(just taut), the influence of ATP on the Ca^{2+}-activated and Ba^{2+}-activated isometric tensions of glycerinated cat right ventricular papillary muscle and the tension transients in response to step stretches in length(less than 1.3 % of Lo within 2 ms) were studied in solution containing in mM:KCl 100, $MgCl_2$ 17, EGTA 5, NaN_3 10, Imidazole 20, Na_2CP 15, CPK 25 units/ml, Na_2ATP 17, 12, 7 or 2, $CaCl_2$ 4.5 (or $BaCl_2$ 1.5) so that pCa=5.2 (or pBa=3.8) at which the isometric tension is maximum at 17 mM Na_2ATP, pH 6.8, temperature 26-27°C. The isometric tension at pCa=5.2 (or pBa= 1.5), which was maximum at 17 mM ATP, was increased by a factor of about 2 with decreasing ATP concentration. The tension transients to step stretch in length(ΔL) was characterized by three distinct phases; an immediate tension increase(ΔT) coincident with the stretch, a rapid exponential tension decrease(τ_1=6-8 ms at 17 mM ATP) and a delayed exponential tension rise(τ_2=90-110 ms at 17 mM ATP). Decreasing ATP concentration increased τ_1 by a factor of about 2 and τ_2 by a factor of 1.5-2.5. The stiffness(ΔT/ΔL) was linearly increased with the increase in tension, similarly both in the Ca^{2+}-activated and Ba^{2+}-activated muscles.

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EFFECT OF CHANGE IN VENTRICULAR CHAMBER GEOMETRY ON VENTRICULAR END-SYSTOLIC PRESSURE-VOLUME RELATION ON CANINE PRESSURE-OVERLOAD HYPERTROPHIED LEFT VENTRICLE. NAKAMURA, T., OKINO, H. and KIMURA, T.* Dept. of Physiology 2, School of Medicine, Tokai University, Boseidai, Kanagawa 259-11

The effect of change in chamber geometry on left ventricular (LV) end-systolic pressure-volume relation was studied in isolated canine left ventricles of 7 normal (N) and 5 pressure overload hypertrophied (H) hearts. In the above preparation, the afterload was changed by a servocontrolled isobaric contraction system, in which the left ventricular ejection pressure (EP) was kept constant during the ejection phase. The LV function was studied at the constant preload, namely at EDP of 20mmHg and the afrerload was changed at intervals of 20mmHg from 160mmHg to 40mmHg in the decreasing order. In H, the occurence of concentric hypertrophy was confirmed by the ratio of end-diastolic volume (EDV) to LV weight (LVW). The EDV/LVW at EDP of 20mmHg in H was 0.32 ± 0.08ml/g (N : 0.68 ± 0.06, $P<0.01$). The slope of EP-ESV (end-systolic volume) /LVW in H was $1.03 \pm 0.16 \times 10^{-3}$ml/g/mmHg (N ; $0.25 \pm 0.52 \times 10^{-3}$, $P<0.01$) and Vd (LV volume at EDP of 0mmHg) /LVW was 0.07 ± 0.02ml/g (N ; 0.25 ± 0.03, $P<0.01$). There is a significant linear correlation between the slope of EP-ESV/LVW and EDV/LVW at EDP of 20mmHg. These results indicated that Emax, which was said to be sensitve to the change in myocardial contractility, was also affected by the change in chamber geometry.

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PRESSURE-VELOCITY RELATION IN THE RABBIT LEFT VENTRICLE. OKUYAMA,H., OKADA,T. AND MASHIMA,H. Dept. of Physiol., Sch. of Med., Juntendo Univ., Bunkyo-ku, Tokyo 113

Pressure vs. ejection velocity relation was determined by isovelocity method in the rabbit left ventricle. The relation can be expressed by a hyperbola similar to the Hill equation, $(P + A)(\dot{V} + B) = B (P_O + A)$, where P is the pressure, $\dot{V}$ the velocity, P_O the maximum pressure, A and B are constants. As the initial volume decreased, A and B, especialy A, increased. Using the thick-walled spherical model, this equation can also be obtained from the Hill equation of wall muscle, $(T + a)(v + b) = b (T_O + a)$, where T is the tension, v the velocity, T_O the maximum tension, a and b are constants. Thus, the constants of the wall muscle, a and b, were calculated from the constants A and B, provided with ventricular volume. Although a and b decreased at smaller volumes, the ratio v_{max}/T_O (= b/a, v_{max} : maximum velocity) was insensitive to a change in the volume. The value of v_{max}/T_O was $1.14 \pm 0.22 \times 10^{-5} cm^2/dyne \cdot sec$ in 9 rabbits. This value sensitively decreased with negative inotropic condition such as decreased heart rate or decreased oxygen supply. Therefore, v_{max}/T_O could be a good index for the ventricular performance based on the contractility of wall muscle.

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MEMBRANE CURRENTS IN SINGLE GUINEA-PIG ATRIAL CELLS.
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Single atrial cells were isolated from the adult guinea-pig heart using a collagenase enzyme method. In normal Tyrode solution quiescent and rod-shaped single cells were 115 um in length and 12 um in width. The cell had following membrane electrical properties: resting potential=-79 mV; input resistance=55 M ; time constant=3 msec; action potential amplitude 117 mV; action potential duration=94 msec and the maximum rate of rise= 263 V/sec. Voltage clamp was carried out by using a one-microelectrode technique. In voltage clamp experiments, a transient inward current, and outwerd background and delayed outward currents were observed on depolarization from the holding potential of -40 mV to various potentials. The transient inward current was abolished by 2 mM Co^{++}, and the outward currents were suppressed by intracellular application of 4-AP. Potassium dependent currents were blocked by intra- and extra-cellular application of Cs^{+}.. The slow inward current inactivation could be separated into fast and slow components. The slow inward current was slightly decreased by 0.1 uM acetylcholine but the kinetics were not modified.

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POST-ACETYLCHOLINE POTENTIATION OF CATECHOLAMINE RESPONSE IN GUINEA-PIG VENTRICULAR MYOCARDIUM. MITSUIYE, T. AND EHARA, T. Dept. of Physiol., Faculty of Medicine, Kyushu University, Fukuoka 812

We have shown that, in K(27 mM)-depolarized guinea-pig ventricular muscle, catecholamine(CA) elicits a small (1-6 mV) depolarization of the resting membrane, and that this CA-induced depolarization(CAD) involves a β-receptor-mediated activation of the membrane slow channel (J Mol Cell Cardiol 15, 1983, in press). We examined the effect of acetylcholine(ACh) on CAD. When ACh was applied to the muscle in the continuous presence of CA, a relaxation of CAD occurred. However, subsequent removal of ACh led to a transient enhancement (rebound) of CAD which peaked in about 1.5 min and subsided thereafter. These phenomena did not occur when atropine or Mn was present but persisted in the presence of phentolamine or hexamethonium. ACh alone had no influence on the membrane potential. ACh exerted effects similar to the above on the histamine- or theophylline-induced depolarizations, in the presence of β-blocker. Under normal conditions, the CA-induced positive inotropy was suppressed by ACh, while removal of ACh caused a transient increase in tension, and this rebound was accompanied by an elevation of the action potential plateau. These results suggest that there is a complex interaction between CA and the muscarinic action of ACh on myocardial cells, besides the wellknown CA-ACh antagonism. The possibility arises that the post-ACh potentiation of CA response involves an increase in I_{si}.

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MEMBRANE CURRENT CHANGE RELATED TO POST-ACh DEPOLARIZATION IN THE CORONARY SINUS CELLS OF CANINE HEARTS. NISHIYE, H., CRANEFIELD, F. P.*, GADSBY, C. D.* Dept. of Physiol., School of Medicine, Juntendo Univ., Bunkyo-ku, Tokyo 113 and The Rockefeller Univ., N.Y.C., New York 10021

The acetylcholine(ACh) evokes a sudden hyperpolarization on an application in coronary sinus cells and sustains the membrane potential to the hyperpolarized level. On sudden removal of ACh, that hyperpolarization declines and is followed by a small depolarization which reaches a peak about 30s after removal of ACh and then decays slowly with an exponential time course of half-time 78s. The current change after removal of ACh(post-ACh current transient) was studied in very small coronary sinus strips by use of a two-microelectrode voltage clamp. Just after ACh removal, steady-state current transiently decreased and then progressively recovered. This post-ACh current transient was inhibited by 2 μM to 5 μM atropine which prohibited ACh-induced current. The amplitude of post-ACh current transient was increased depending on the concentration of applied ACh and the duration of application. That current transient was prominently increased by coexistence of catecholamine. The reversal potential of post-ACh current transient was as same as the equilibrium potential for K ions. Therefore, the post-ACh current transient is concluded to be due to a transient reduction in steady-state K conductance.

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EFFECT OF ACETYLCHOLINE ON THE INITIATION OF CIRCUS MOVEMENT TACHYCARDIA. Sawanobori, T., Hirano, Y., Adaniya, H., Hirota, A., Fujii, S., Sakai, T. and Kamino, K. Dept. of Cardiovasc. Dis., MRI and Dept. of Physiol., Tokyo Med. and Dent. Univ., Bunkyo-ku, Tokyo

Ring-shaped preparations of bullfrog atrium (1.0 - 1.5 x 1.5 - 2.0 cm) containing the ostium of sinus venosus excluded pacemaker area were exposed to Ca^{2+}-free solution after staining with voltage sensitive merocyanine-rhodanine dyes (Dye XVII or NK2761). Spread of activation wave fronts during basic and premature stimuli was optically recorded simultaneously from eight different portions of the preparation. In Ca^{2+}-free solution, circus movement tachycardia (CMT) was hard to be evoked because of long action potential durations (APDs). Addition of acetylcholine (Ach) (10^{-10} - 10^{-7} g/ml) shortened APDs in proportion to concentration of Ach within 2 min and displayed a disparity of APDs, especially in lower concentration of Ach. CMT was initiated by the application of a single timed premature stimulus delivered to the site showing non uniformity of APDs. The rate of tachycardia decreased markedly at initial stage and reached at the plateau after about 100 beats. CMT was terminated spontaneously after showing fluctuation of beat to beat interval or by a timed stimulus given between 47 to 78% of the preceeding cycle length. Such an optical method can improve our understanding of conduction abnormalities.

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FUNCTIONAL CHARACTERIZATION OF PACEMAKING ACTIVITY OF PERIPHERAL, TRANSITIONAL AND CENTRAL CELLS OF RABBIT SINUS NODE. KODAMA, I., BOYETT, MR., TOYAMA, J. AND YAMADA, K. Dept. of Circulation and Respiration, The Research Institute of Environmental Medicine, Nagoya University, Chikusa-ku, Nagoya 464 Japan, Dept. of Physiology, The University of Leeds, Leeds LS2 9NQ, England

Small specimens of tissue having a diameter of about 0.3 mm were isolated from peripheral, transitional and central area of rabbit sinus node, and superfused with Krebs Ringer's solution at 33°C. In response to premature electrical stimuli, peripheral (P) cells near the crista-terminalis showed the earliest recovery of excitability during diastole, while central (C) cells showed the slowest one. Intermediate results were obtained in transitional (T) cells. The decrease of maximum diastolic potential (MDP), amplitude (AMP) and upstroke velocity ($\dot{V}max$) of action potential following a sequence of rapid stimulation (overdrive) were more remarkable in C cells than in T and P cells. The depressant effect of high K^+ on pacemaking activity was most significant in C cells. Thus, all the preparations of C cells became quiescent under 14 mM K^+, while 20 % and 60 % preparations were still beating in cases of T cells and P cells respectively. The negative chronotropic effect of acetylcholine (Ach) was greater in P cells, while C cells were most resistant to that. These differences of pacemaking activity among P, T, and C cells may contribute to the pacemaker shift in the sinus node.

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OPTICAL MONITORING OF PACEMAKER FUNCTION IN CARDIA BIFIDA OF EARLY CHICK EMBRYO. FUJII, S., HIROTA, A., SAKAI, T. and KAMINO, K. Dept. of Physiology, Tokyo Medical and Dental University School of Medicine, Bunkyo-ku, Tokyo 113

Using an optical method for monitoring membrane potential in embryonic prebeating chick hearts at the 7-9 somite stage of development, we have found that when the early embryonic prebeating hearts were separated into right and left, the synchrony and the intrinsic rhythmicity in spontaneous action potential activity remained independently, in each part. For solving certain problems in genesis of early development of cardiac functions, embryonic double-hearts produced experimentally have been used. For additional information in natural preparations, it would be greatly advantageous if one could use "cardia bifida" embryo formed congenitally. During experiments on early embryonic chick hearts, we found the congenital double-hearted embryos. In these cardia bifida embryos produced experimentally or congenitally, the right and the left half hearts were tubulous, and in both, spontaneous rhythmical action potentials and beating were detected. There were differences in the rhythm between the right and the left half hearts. We also have monitored optically action potentials in the malformed embryonic heart formed by partial fusion of the primordia. The results are discussed in relation to the genesis of intrinsic pacemaking activity in cardiac primordia and to spatial gradient of rhythmicity in the early phases of cardiogenesis.

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OPTICAL REVEALING OF PACEMAKING BEHAVIOR IN EARLY EMBRYONIC CHICK HEART. HIROTA, A., SAKAI, T., FUJII, S. and KAMINO, K. Dept. of Physiology, Tokyo Medical and Dental University School of Medicine, Bunkyo-ku, Tokyo 113

The propagation of spontaneous action potentials in 7-9 somite embryonic pre-contractile chick hearts was measured optically using a potential-sensitive merocyanine-rhodanine dye. Spontaneous optical signals, corresponding to action potentials, were recorded simultaneously from 8-16 different sites of the primitive embryonic heart. Short delays were observed in the time of occurrence of optical signals obtained from the different regions. We have found (i) switching phenomena: the site exhibiting pacemaking priority was first situated in the right pre-atrium, thereafter it switched over to the left pre-atrium, or vice versa, and (ii) double pacemakers: two different pacemaking areas were situated independently in the right and left pre-atrial portions of the heart. On the basis of analysis of such behavior, it was concluded that the regional priority of the pacemaking activity is not rigid but is flexible, and that the direction of the spread of excitation is adaptable to the circumstances in the early embryonic heart. We concluded that during the early phases of cardiogenesis, there exists a labile stage of regional priority of pacemaker function, and with the development of the regional gradient of rhythmicity, a single pacemaking area is organized.

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EFFECT OF GRAYANOTOXIN (GTX) ON THE RABBIT SINO-ATRIAL (S-A) NODE CELLS (II). NAKAO, M. and SEYAMA, I. Dept. of Physiol., Hiroshima Univ. Sch. Med., Hiroshima 734

GTX (10 μM) suppressed the spontaneous activity of S-A node cells with depolarizing the membrane. The following application of 1 μM of tetrodotoxin (TTX) restored the spontaneous activity. The voltage clamp experiments on a small S-A node specimen in GTX medium revealed that the maximum conductance of slow inward current was reduced by 15% and the inactivation curve of fast inward current was shifed in the hyperpolarized direction without affecting outward current. Most significant effect of GTX was to induce the time independent current (I-GTX), which was linear to the membrane potential. When the parameters regarding these changes were incorporated into the model developed by Yanagihara et al.(1980), the phenomena induced by GTX were well simulated. By comparing the contribution of each change in parameters to the pattern of simulated action potential, it may well be concluded that I-GTX is mainly responsible for the sinus arrest produced by GTX.

At higher concentration (100 μM), GTX suppressed all time dependent current systems and the time independent current became dominant. These findings suggested whether the intracellular ionic milieu was drastically changed or the ionic channels were modified.

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EFFECTS OF Cs^+ ON THE RABBIT SINOATRIAL NODE CELLS. GOTO,K., TAKAHASHI,T., SUDO,S., MIYAMAE,S. AND NAKANO,S. Dept. of Physiology, Kanazawa Medical University, Uchinada, Ishikawa, 920-02

The effects of Cs^+ on the rabbit sinoatrial node cells were studied, using conventional microelectrode techniques. Hyperpolarizations induced by Cs were recorded from the preparations which were perfused previously with an extracellular K-free solution. The Cs induced hyperpolarizations after K-depletion depended on Cs-concentration in the range of 2 to 30mM.

When 5mM Cs was used as an activator, a subthreshold oscillation appeared and was followed by arrhythmic action potentials which were smaller than with the standard solution. As the concentration of Cs increased, the Cs activated hyperpolarization increased, but the following action potentials showed the maximal response at 10mM Cs.

The hyperpolarization were considered to be electrogenic. However the arrhythmic action potentials suggest the posibility that Cs reduces the background current, abolishes the K^+ depletion process, and causes the pacemaker-shift.

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EFFECTS OF ACETYLSTROPHANTHIDIN ON CHANGES OF CABLE PROPERTIES IN THE CANINE PURKINJE FIBERS. Endou, K. Dept. of Medicine, Kitasato University, Sagamihara, Kanagawa, 228

Canine Purkinje fibers were perfused with 4 mM K Tyrode solution bubbled with 95% O_2 and 5% CO_2. The bath was separated to two chambers with a plastic plate. Constant currents (1-4 x 10^{-7} A) were flown extracellularly from one chamber for 150 msec. Electrotonic potentials were obtained by two microelectrodes inserted in the Purkinje strands in another chamber. Space constant (λ) and internal resistance (r_i) were measured under the control condition and during Acetylstrophanthidin (AS). With 10^{-6} g/ml AS, λ increased by 75 ± 18% (mean ± SE, n=4) compared to the control, wherease r_i decreased 44 ± 4%. These changes were significantly different from changes in λ (33± 10%, n=6) and r_i (23 ± 6%, n=6) with the low doses of AS (10^{-10} to 10^{-11} g/ml). With the middle doses (10^{-7} to 10^{-8} g/ml AS) λ increased by 40 ± 6% (n=5) and r_i decreased by 37 ± 3% (n=5). The results demonstrate clearly that the changes of r_i were depedent on AS concentration.

The decrease in r_i may not be explained by stimulation of Na-K pump by AS, because Deitmer reported that intracellular Na ion activity increased by 10^{-6} M strophanthidin.

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TWO COMPONENTS OF THE TRANSIENT OUTWARD CURRENT IN RABBIT CARDIAC PURKINJE FIBERS. GOTO, J. and COLATSKY, T.J.* Dept. of Circulation and Respiration, Res. Inst. of Env. Med., Nagoya Univ., Nagoya 464 and Dept. of Physiology, Cornell Univ. Medical College, New York, NY.*

The transient outward current (I_{to}) was investigated in short rabbit Purkinje fiber using the two-microelectrode voltage clamp technique. I_{to} was activated by depolarizations positive to -20 mV and could be separated into two kinetically and pharmacologically distinct components; an initial brief component which was inhibited when external NaCl was replaced by Na isethionate, and a second slower component which was insensitive to Cl-removal. The early component peaked within 8 msec and decayed with a time constant of 2-4 msec at 0 mV. The reversal potential was -67 mV, which is close to the equilibrium potential of Cl indicated by ion selective electrode measurements. The second component peaked later (~20 msec) and decayed more slowly (τ =10-15 msec); it could be specifically inhibited by 5-10 μg/ml quinidine and the reversal potential was -86 mV, which is close to the equilibrium potential of K and the current-voltage relationship showed the inward rectification beyond -65 mV. In conclusion, two components of I_{to} were identified, one was Cl-sensitive current and the other was quinidine-sensitive current which was suggested to be carried by K.

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RECOVERY OF Ca CURRENT FROM INACTIVATION: THE ROLES OF Ca INFLUX, MEMBRANE POTENTIAL AND CELLULAR METABOLISM.
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Ca currents (I_{Ca}) separated from other membrane currents were examined with regard to their recovery from inactivation in voltage-clamped, internally perfused isolated nerve cell bodies of Helix aspersa. Cells were stimulated by two depolarizing pulses separated by variable intervals after a prepulse varing in duration. Ba currents (I_{Ba}) and I_{Ca} were compared. Recovery of I_{Ca} was fit by two exponentials and the τ's of the recovery were increased when prepulse duration was prolonged or extracellular Ca concentration was increased. I_{Ba} recovery followed two exponentials and revealed reversible inactivation gating of potential-dependent membrane Ca channels. Intracellular perfusion with EGTA resulted in less inactivation and faster recovery for I_{Ca} but not for I_{Ba}. The recovery rate of I_{Ca} was faster in high pH and slower in low pH. Intracellular ATP increased the rate of recovery and the Ca-ATPase inhibitor, vanadate inhibited the recovery. It was concluded that recovery of I_{Ca} depends both on Ca influx and membrane potential and is modulated by cellular metabolism.

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Ca-DEPENDENT OUTWARD CURRENTS IN FROG MYOCARDIUM.
GOTO, M., HYODO, T. AND IKEDA, K. Dept. of Physiol., Faculty of Medicine, Kyushu University.

Properties of Ca-dependent outward current were studied in bullfrog atrial muscle using double sucrose-gap techniques. Decreasing $[Ca]_o$ decreased the instantaneous outward current (I_{k1}) and inward background current (I_b), whereas increasing $[Ca]_o$ increased the curresnts. When g_{k1} was blocked with 0.3 mM Ba, these effects were absent. In the presence of Ba and TTX (1.5×10^{-6}M), 3 mM Co abolished the slow inward current (I_{si}) as well as I_{si}-dependent tension, and depressed the delayed outward current (I_{out}). The depression was confined to the voltage range of activation of I_{si} and I_{si}-dependent tension. Isoproterenol (10^{-7}M) increased the I_{si} and I_{out} at the same voltage range. Activation of I_{out} became fast while deactivation became slow by isoproterenol and opposite effects were produced by Co. In the presence of Ba, TTX and Co, Ca-free EGTA (0.3 mM) Ringer further depressed the I_{out}, eliminating the remained tension response. The depression, however, was stronger, the stronger the depolarization. The activation of I_{out} was markedly retarded and the amplitude of fully activated I_{out} diminished, while the deactivation became faster. Thus, Ca-dependent outward current was classified into 1) Ca-linked I_{k1}, 2) I_{si}-dependent I_{out} and 3) I_{si}-independent but Ca-dependent I_{out}.

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INFLUENCE OF INTERNAL Ca ON THE SINGLE Ca CHANNEL CURRENT IN VENTRICULAR CELLS.
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Unitary currents of Ca channels were recorded by the patch clamp technique from the membrane of ventricular cell isolated from guinea-pig heart. The external solution at the patch membrane was Na-free and K-free and contained 50mM Ba and 30μM TTX. Short(1-5msec) inward current pulses with the amplitude of 0.5-1 pA and with the slope conductance of about 10 pS were elicited by the depolarization clamp steps to 50-100mV positive to the resting potential in the normal Tyrode. The opening probability was increased and the pulses appered often in a burst of several tens of ms with increasing the depolarization. The average of the single channel currents showed a time-dependent inactivation as the slow inward current. Single Ca channel currents were also recorded in the presence of 140mM K and 1.8mM Ca only by shifting the holding potential and depolarization level by 70mV to less positive potentials. The increase in the intracellular Ca in contracting cells under such condition did not affect much the opening probability of Ca channels. Similar single Ca channel currents could be recorded in inside-out patch exposed to this high K, Ca-containing solution. It indicates that Ca channels can remain in an available state in spite of the presence of high Ca near the intracellular surface of Ca channels.

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DISTRIBUTION OF INWARD RECTIFIER K^+ CHANNEL IN RABBIT HEART. Kiyosue, T., *Soejima, M*, Noma, A**and Irisawa, H** Dept. of Physiology, Medical College of Oita, Oita 879-56. *Dept. of Internal Medicine, Jikei University School of Medicine, Minato-ku, Tokyo 105.**National Institute for Physiological Science, Myodaiji, Okazaki 444.

Ventricular, atrial, A-V and S-A nodal cells were isolated by perfusing rabbit heart with collagenase. We estimated values of specific membrane resistance (Rm) around the resting potential in the whole cell voltage clamp. These were 0.75KΩcm² for the ventricular cell and 12KΩcm² for the A-V and S-A nodal cells. To elucidate the ionic basis of the difference in Rm, we recorded the activity of single K^+ channels by patch clamp method. Ventricular cells had a higher density of inward rectifier K channels than the pace maker cells. This channel had a large unit conductance (45pS in 150mM K^+) and a high open probability near the resting potential. The mean current value obtained by averaging the current through a membrane patch is 20-30 times larger in ventricular cells than in pace maker cells. These results suggest that low resting potential in pace maker cells is explained by the lack of inward rectifier K^+ channels.

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LOW K^+-INDUCED AFTERDEPOLARIZATIONS AND REPETITIVE RESPONSES. HIRAOKA, M., KAWANO, S. AND HIRANO, Y. Dept. Cardiovasc. Dis., MRI, Tokyo Med. and Dent. Univ., Bunkyo-ku, Tokyo 113

Depolarizing afterpotentials play significant roles for abnormal impulse formations of cardiac muscle. They are divided into two and called early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs). When $[K^+]_o$ was reduced to 1 mM, guinea-pig ventricular muscles developed DADs and triggered-tachycardias in response to train impulses. At the same time, EADs became prominent and second upstrokes appeared in 19 of 66 fibers. Abnormal excitations arising on EADs were dependent on $[Ca^{2+}]_o$. They became prominent at 3.6 mM-Ca^{2+} and appeared repetitively. These activities were not suppressed by verapamil. Repetitive responses had a mean frequency of 30.1 ± 6.7 (S.D) Hz and Vmax of spikes exceeded 100 V/sec. During repetitive responses, the developed tension became tetanic. Replacement of Sr^{2+} or Mg^{2+} for Ca^{2+} stopped the activities. Repetitive activities were dependent also on $[K^+]_o$, being appeared at 1 mM-K^+ or less, and abolished in 2 mM-K^+ or Rb^+. The spike genesis of repetitive responses were dependent on $[Na^+]_o$ and blocked by tetrodotoxine. The results indicate that suppression of the Na^+-K^+ pump by low $[K^+]_o$ causes an accumulation of $[Na^+]_i$ and $[Ca^{2+}]_i$ may induce an alteration of fast Na^+ current, which forms the basis of rapid and repetitive firings.

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THE EFFECT OF EXTRACELLULAR pH ON THE Na CURRENT IN ISOLATED, SINGLE, RAT VENTRICULAR CELLS. -VOLTAGE CLAMP STUDY-

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The effect of extracellular pH on the Na current (I_{Na}) in enzymatically isolated single rat ventricle cells was examined. A suction pipette was used for passing current and internal perfusion. The membrane potential was measured by a microelectrode. Potassium currents were blocked by replacing K with Cs in the internal and external solutions. The external Na concentration was reduced to 40 mM and temperature 23°C to ensure good voltage control. Low pH (6.4-4.5) produced a marked inhibition of I_{Na}. The voltage-current relationships of I_{Na} were shifted to more positive potentials without changing in the reversal potential (E_{Na}). The steady-state inactivation curves of I_{Na} (h_∞) also shifted to the same direction. High pH (9-10) shifted the voltage-current curves and h_∞ to more negative potentials. The rates of the inactivation and recovery from inactivation of I_{Na} were reduced by low pH. It was concluded that extracellular pH affects the Na channels in single isolated rat heart cells in a similar way as in squid axon and in the myelinated nerve of the frog.

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EFFECT OF CHANGE IN [Na]o ON THE ELECTRICAL PROPERTIES AND ACTION POTENTIAL OF SINGLE FROG VENTRICULAR CELL.

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Single ventricular cells were isolated from an adult bull frog by perfusion of the heart with collagenase and trypsin. The suction pipette method was used for recording the membrane potential. Resting potential, amplitude, and maximum rate of rise of the action potential were estimated to be -75.3±2.2 mV, 115.7±7.2 mV, 52.0±13.0 V/sec (N=15), respectively. The membrane characteristics were determined to be 1.80±0.34 kΩ cm^2 for Rm and 1.30±0.52 μF/cm^2 for Cm. Since the diameter of the suction pipette was large enough to dialize the cell, one can keep the intracellular ionic concentration constant, even in the environment where extensive ionic movement across the membrane may be induced through various ion exchange mechanisms. Among these ion exchange systems, Na-Ca exchange mechanism plays an essential role on preserving ionic gradient across the membrane. Thus, it has been examined what effect on the venticular action potentials the reduction of [Na]o produceS.When used the large diameter pipette, the amplitude of action potential did not change and the configuration of cell was held normal even in the 30% [Na]o medium, suggesting that the ionic environment in the cell keep constant, in spite of massive movement of Ca with Na-Ca exchange system.

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EFFECT OF SODIUM DEPLETION ON THE TENSION OF FROG VENTRICULAR MUSCLE.

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Frog ventricular muscles were bathed in Na^+-free solution (Tris-Cl or LiCl Ringer) for 1-2hrs. The content of Na^+ was measured to be about 50 μmol/kg w.w. (Na^+-depleted muscle). KCl- and voltage clamp-induced depolarization produced tensions with very slow relaxation. Tension-voltage relation changed from a monotonically increasing relation observed in Na^+-rich solution (more than 20mM) to a bell-shaped curve. In Na^+-depleted muscles tension peaked around 0mV and decreased back to baseline tension around +70mV. The voltage-dependence of tension in Na^+-depleted strips was similar to that of TTX-insensitive slow inward current (Isi). The effect of pharmacological interventions on the tension-voltage and I-V relation was similar. These results suggest that the tension in Na^+-depleted strips is correlated with the activation of Isi. There is, however, discrepancy between the time course of Isi and that of tension. This result indicates that Ca-channel may inactivate more slowly (τ= around 2 sec).

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THE MECHANISM OF RHYTHMIC HYPERPOLARIZATION IN HAMSTER SUBMANDIBULAR GANGLION CELLS.
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The mechanism of spontaneous and caffeine induced rhythmic hyperpolarizations (HPs) which occur in hamster submandibular ganglion cells were further analyzed pharmacologically by intracellular recording. Intracellular injected EGTA blocked the generation of caffeine rhythmic HPs. Dantrolene sodium incompletely abolished the generation of caffeine rhythmic HPs. Externally applied ruthenium red incompletely abolished the generation of spontaneous rhythmic HPs. Perfusion with Co^{2+}- or Mn^{2+}-containing saline completely inhibited the generation of caffeine rhythmic HPs. The inhibitory effects of both ions appeared slowly and continued for a long time. It was confirmed that perfusion with Ca^{2+}-free, Mg^{2+}-substituted saline did not prevent caffeine rhythmic HPs but rather increased the frequency of the rhythmic HP. However, the amplitude did not change in this experiment. The abolishment of the rhythmic HPs by 4-aminopyridine and Ba^{2+} provided new evidence showing that these potentials are generated by the activation of $G_{K(Ca)}$. The results of this study suggest that the mechanism of the rhythmic HPs in this ganglion cell consists of three steps: 1) the passive influx of external Ca^{2+} through the resting plasma membrane; 2) the uptake and release of Ca^{2+} at an internal Ca^{2+} reservoir; 3) the activation of the $G_{K(Ca)}$ in the plasma membrane by an increased $[Ca^{2+}]i$.

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CHANGE OF KCl-RESPONSE AFTER APPLICATION OF METHIONINE-ENKEPHALIN IN AUTONOMIC GANGLIA.
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In the isolated superior cervical ganglia of guinea pigs, KCl-induced depolarization (KCl-response) was examined before, during and after short application of methionine-enkephalin (M-enk). KCl-response was slightly depressed immediately after application of M-enk. Then the responses were augmented dose-dependently up to two times during one or more than one hour of washout period, returning finally to the control level. The augmentation was antagonized by the same doses of naloxone. Even in the presence of hexamethonium or in low Ca^{++} solution, KCl-response changed in the same manner. Postsynaptic potential was also depressed by M-enk and enhanced by removal of the drug, although the depolarizations evoked by Ach were not changed. Resting membrane potential and the impedance were scarcely affected. The magnitude of the positive after-potential of action potential was enhanced by application of M-enk. In K-free solution, M-enk hyperpolarized the membrane potential and prolonged the after-potential.

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EFFECTS OF DILTIAZEM ON SYMPATHETIC NERVE-MEDIATED CONTRACTIONS OF VAS DEFERENS IN VITRO.
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In the isolated guinea pig vas deferens, tetanic stimulation of intramural nerves produced a biphasic contraction , a phasic early component followed by a tonic, second component. Diltiazem (10^{-5} M) reduced the second component selectively. This drug at the same dose, however, did not affect the contractile response to exogenously applied noradrenaline (NA) and direct, electrical stimulation of smooth muscles. Phentolamine suppressed the tonic component of nerve-mediated contractions. Thus, it may be concluded that diltiazem (10^{-5} M) has an inhibitory effect on NA release from the nerve terminals. It was also observed in the epididymal portion of rat vas deferens that diltiazem as well as verapamil and nifedipine markedly reduced the second component of a biphasic contraction by tetanic stimulation. On the other hand, the contraction by single stimulation was not affected in the epididymal portion but augmented by 80% in the prostatic portion after application of diltiazem.

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EFFECTS OF VIP AND NALOXONE ON THE INHIBITORY JUNCTION POTENTIALS IN THE DUODENAL SMOOTH MUSCLE CELLS OF THE GUINEA-PIG. OHKAWA,H. Dept. of Physiology, Yamaguchi Univ. Sch. of Med., Ube 755

The membrane potential of the duodenal smooth muscles was not affected by VIP (1.7×10^{-11}-1.7×10^{-7}M). The amplitude and the other parameters on the i.j.p. were not changed in VIP used. Met-enkephalin had no effect on the membrane potential and the i.j.p.. The membrane was depolarized by ß-endorphin. The amplitude was slightly reduced and the spontaneous e.j.p. was generated by ß-endorphin.

Naloxone hyperpolarized the membrane. At high concentration, the inhibition of the i.j.p. was observed. The membrane potential was slightly decreased in levallorphan. Levallorphan reduced the amplitude of the i.j.p.. The spontaneous e.j.p. generated in a certain population of the cells. It is concluded that VIP, Met-enkephalin and ß-endorphin were not the non-adrenergic inhibitory substance. Endorphin is relate with the excitatory control mechanism on the intestinal motility.

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SLOW DEPOLARIZING SYNAPTIC POTENTIAL AND CHEMOSENSITIVITIES OF NEURONS IN THE ISOLATED SUBMUCOUS PLEXUS OF THE GUINEA-PIG CECUM. MIHARA, S., *KATAYAMA, Y., and NISHI, S. Dept. Physiol., Kurume Univ. Sch. Med., Kurume and *Dept. Autonomic Physiol., Med. Res. Inst., Tokyo Med. & Dent. Univ., Tokyo.

Intracellular recordings were made in vitro from single neurons of the submucous plexus of the guinea-pig cecum. The slow EPSPs, which were not affected by cholinolytic agents, were observed in about 90 % of neurons following a tetanic focal stimulation. Electrophysiological analysis revealed that there are two types of slow EPSP differed in ionic mechanism. In a majority of neurons the slow EPSP was brought about by suppression of the M-channels, while in a minority of neurons the slow EPSP was generated by depression of the resting G_K. The neuroactive substances known to be contained in the submucous plexus, such as 5-HT, ATP, and several peptides, were applied by pressure ejection onto the plexus neurons. All of these substances exerted depolarizing actions on the neurons. The 5-HT- and CCK-induced depolarizations appeared to be due to an increased conductance to Na^+ and K^+. The ATP-elicited depolarization was due to suppression of the M-channels, while SP-, VIP-, SOM-, and NT-produced depolarizations were due to depression of the resting G_K. The results raised the possibilities that ATP might mediate the slow EPSP of M-channel type and that the neuropeptides except for CCK might induce the slow EPSP of the resting G_K type.

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EFFECTS OF CALCIUM IONS AND N-(6-AMINOHEXYL)-5-CHLORO-1-NAPHTHALENESULFONAMIDE (W-7) ON THE CELL JUNCTIONAL PERMEABILITY IN THE CULTURED AUERBACH'S PLEXUS CELLS. MARUYAMA, T. Dept. of Applied Physiology, Tohoku University School of Medicine, Sendai, 980

The cultured Auerbach's plexus cells which were derived from the small intestine were studied in this experiment. The cells proliferated well the cultured system. During the culture, the Auerbach's plexus cells connected with each other, some by their processes and the other by cell bodies. These cells are coupled by low resistance channels that permit the cell-to-cell passage of the electrical current and florescein dyes. The permeability of the junctional membrane channels is regulated by the cytoplasmic cencentration of calcium ions. Raising of calcium ions into cell, the junctional conductance markely fallen and the transfer of florescein dyes also blocked. W-7, a specific inhibitor of calmodulin, blocked the electrical communication, and inhibited the transfer of florescein dyes for the junctional membrane channels or processes of their cells.

The results indicate that calmodulin plays a major role in modulating the effects of calcium ions on the junctional membrane channels and the processes in the cultured Auerbach's plexus cells.

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ACTIONS OF VARIOUS BRAIN-GUT PEPTIDES ON NEURONS FROM RAT BRAINSTEM CULTURES.
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Using cultures of dissociated neurons from the lower brainstem of 14-15-day-old rat embryos, we studied a specific relation between neuronal activities of five types of neurons (oval, fusiform, polygonal, pyramidal and multipolar shapes) and their sensitivities to various brain-gut peptides (motilin, secretin, VIP, CCK-8, CCK-4, neurotensin, somatostatin and bombesin). Among others, motilin and bombesin showed transmitter-like actions. Motilin induced a facilitatory response in all types of 24 neurons out of 55, but the other 8 neurons showed a strong inhibitory response with the threshold dose being as low as 10-15 nA. Bombesin induced both facilitatory and inhibitory response in polygonal and multipolar neurons, a facilitatory response in fusiform and pyramidal neurons, and an inhibitory response in oval neuron (1/10). The bombesin-induced facilitatory response has a lower threshold (5-10 nA). Both motilin and bombesin action appears to be transmitter action, but it is commonly slow in onset (2-5 s). Therefore, it is remotely possible that they are transmitters. Both VIP and CCK-8 showed non-effective response. The other peptides induced facilitatory and/or inhibitory responses with very slow onset.

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CHEMO- AND BARORECEPTORS IN THE AORTIC TRUNK OF THE TOAD.
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A fine branch of the vagus(aortic nerve), 50μ in diameter, terminates in the aortic trunk in a limitid area joining the pulmocutaneous artery. Afferent and efferent impulses were induced on this fine nerve. Efferent discharge originated mainly from the sympathetic, partly from the parasympathetic. Most of the afferent impulses were chemosensory; they responded typically to the flow stop, O_2-lack, CO_2-excess, NaCN and Ach. Histologically fluorescent cells were confirmed to be located in the muscular layer of arterial wall. Fine structure was similar to that of the glomus cell in the carotid labyrinth. Intimate connection was observed between the cell and smooth muscle as has been reported in Xenopus labyrinth. Baroreceptor impulses were also induced. With the threshold pressure in the range of normal fluctuation of blood pressure, they were relatively slow adapting. Firing synchronous with the heart beat was confirmed in the nerve strand split from vagus branch. In anesthetized toad administration of NaCN elevated blood pressure and accelerated respiration. Electrical stimulation of the central cut end of the aortic nerve evoked the same type of reflex response. Therefore, the aortic trunk in toads functions as a reflexogenic area in controlling the circulation and respiration. Chemoreceptor function is predominant there.

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NALOXONE EFFECT ON EVOKED POTENTIALS IN THE CAT SOLITARY TRACT NUCLEUS BY STIMULATION OF THE CAROTID SINUS NERVE.
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Using a fiber-filled double-barrel coaxial electrode, we studied naloxone effect on the carotid sinus nerve (CSN) terminals in the solitary tract nucleus (NTS) and on the CSN cardiovascular reflex. Averaged field potentials were evoked in the dorsal medulla by stimulation of the CSN, and sites of the maximal potentials were identified in electrode tracks. Naloxone was iontophoretically injected to determine whether evoked potentials were reduced or not. Since we obtained evidence that a large amount of naloxone can block impulse conduction in the CSN afferent fibers, we used a smaller amount of naloxone. The sites, where reduction of the potentials was induced by the application of naloxone, were marked with HRP reaction products. These sites were distributed in the dorsal motor nucleus of the vagus, medial, lateral and dorsal subnuclei of the NTS. These results suggest that opiate may be a synaptic transmitter released from the CSN terminals in the above-mentioned sites.

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CATECHOLAMINE-RELEASING ACTION OF TEA FROM CARDIAC SYMPATHETIC NERVE TERMINALS. UEHARA A. IMANAGA I. MIYASE T.* SAKAMOTO Y. Dept. of Physiology, Sch. of Medicine, Fukuoka Univ., 45-1-7, Nanakuma, Jonan-ku, Fukuoka 814-01, Japan

Tetraethylammonium (TEA) ion exerted dose-dependently such effects on isolated canine right ventricular muscle as a shortening of action potential duration (APD), an increase in action potential plateau, an increase in twitch tension and a shortening of time to peak tension, when it was applied extracellularly. These effects of TEA were reversible, and were abolished by β-receptor blockers and reserpinization. These effects of TEA were dependent on extracellular Ca^{++} concentration, did not show tachyphylaxis, were abolished by TTX preincubation and were about 300 times less potent than those of Tyramine. Catecholamine like effects of Tyramine were independent on extracellular Ca^{++} concentration, showed tachyphylaxis and were insensitive to TTX. Intracellular applied TEA produced a prolongation of myocardial APD which was caused by K^{+}-channel inhibitory action of the drug. Fluorescent photomicroscopic finding showed that the preparation was densely innervated by sympathetic nerve fibers. Extracellularly applied TEA prolonged APD of isolated postganglionic sympathetic neuron. These results suggest that TEA releases catecholamine from sympathetic nerve terminal by acting on the sympathetic neuron, and that mode of catecholamine releasing action of TEA is different from that of Tyramine.

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EFFECT OF CUTANEOUS AND BARORECEPTOR AFFERENT STIMULATION ON ADRENAL SYMPATHETIC EFFERENT UNITARY ACTIVITY. ITO, K., KIM, P., SATO, A. and SATO, Y. Dept. of Physiology, Tokyo Metro. Inst. Gerontol., Itabashiku, Tokyo 173

It has been reported that cutaneous or baroreceptor afferent stimulation produces reflexly changes in adrenal sympathetic efferent mass discharge activity in anesthetized rats. The purpose of the present experiment is to analyze the response pattern of these individual sympathetic unitary activities in the anesthetized rats. In the case of noxious pinching stimulation of the lower chest skin for 1 min, activities of most single fibers were facilitated, while those of a few fibers were inhibited. In the case of non-noxious brushing stimulation, activities of about a half of single fibers were inhibited, while those of another half were slightly facilitated. In the case of baroreceptor stimulation, activities of most fibers were markedly inhibited. It was further noted that a single sympathetic neurons receives plural afferent inputs from the different stimulations used, and that activities of many single fibers were inhibited more dominantly by baroreceptor stimulation than the cutaneous brushing stimulation.

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REFLEX EFFECT OF CUTANEOUS STIMULATION ON THE SECRETION OF ADRENAL MEDULLARY DOPAMINE. KUROSAWA, M., NAKAMURA, H., SATO, A. and SHIMAMURA, K. Dept. of Physiology, Tokyo Metropolitan Inst. of Gerontology, Itabashiku, Tokyo 173

It is well known that epinephrine (E) and norepinephrine (NE) are secreted from the adrenal medulla. We have shown that the secretion of adrenal medullary E and NE is influenced reflexly by noxious and non-noxious mechanical cutaneous stimulations through the central nervous system (CNS) via the adrenal sympathetic efferent nerve in anesthetized rats. Recently, it was shown that dopamine (DA) is secreted from the adrenal medulla. Therefore, in the present experiment, the effect of the cutaneous stimulation on adrenal medullary DA secretion was studied in anesthetized CNS intact rats. Reflex response of secretion rate of DA was similar to those of E and NE. For examples, noxious pinching stimulation of the lower chest skin for 3 min produced increases in DA, E and NE secretion rates, and non-noxious brushing stimulation produced decreases in all secretion rates.

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EFFECTS OF THERMAL AND NOXIOUS STIMULI ON CARDIOVASCULAR FUNCTIONS IN ANESTHETIZED RATS

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Changes in heart rate (HR) and arterial blood pressure (BP) induced by cutaneous thermal and noxious stimuli were compared in rats anesthetized with urethane and pentobarbital. Wistar adult rats were kept in a moderate anesthetic condition, in which any behavioral signs such as wink, chewing and whisker tic lik movement, except the corneal reflex were visually observed during the experiment. A rectal temperature was kept constant at 37.0°C - 38.5°C. Scrotal skin was thermally stimulated by using a water perfused thermode of its water temperature at 38°C. A proximal portion of the tail skin was mechanically stimulated by a noxious pinching. (1) Increases in HR and BP were observed during scrotal skin heating in rats anesthetized with urethane, but decreases in HR and BP in rats anesthetized with pentobarbital. (2) Though increases in HR and BP were induced by noxious pinching in rats with urethane, decreases in BP and in general, increases in HR were observed in rats anesthetized with pentobarbital. From the present experiment, it was confirmed that the antagonistic responses of the cardiovascular functions in the state of these two different kinds of anesthesia are elicited not only by thermal stimuli on the scrotal skin but also noxious stimuli on the tail skin in the rat.

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FLUCTUATION OF RESPIRATORY PERIODIC DISCHARGE IN THE RENAL SYMPATHETIC NERVE ACTIVITY.

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The phase relation of respiratory burst discharges in the renal sympathetic nerve activity to those in the phrenic nerve activity were investigated by means of pulse weight cross-correlation analyses. Experiments were performed on vagotomized 37 rabbits being anesthetized with ether, immobilized and ventilated artificially under monitoring end-tidal CO_2 level. Depressor nerves were cut. Accompanying with changes in the arterial pressure level the phase relation of the initiating point of renal respiratory volley to that of phrenic volley varied more markedly than as the ceasing point did. The time interval from the initiating point of renal respiratory volley to that of the nearest phrenic volley was prolonged simultaneously with an elevation of the arterial pressure as the sinus nerve was intact, while this time interval was shortened antecedently to a spontaneous elevation of the arterial pressure after severance of sinus nerves. When the respiratory period was elongated (1.1 to 2.4 sec) by the change in ventilation (F_{ECO_2}: 2.6-5.3%) irrespective to the intactness of the sinus nerves, the phase relation of renal respiratory volleys in the respiratory cycle shifted successively in the following order: expiratory(E)-inspiratory(I) phase-spanning, E, I-E phase-spanning, I and E-I (or E-I-E) phase-spanning phase. These results suggest that the central respiratory rhythm may play some role in the neural vasomotor control.

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CEREBELLAR VERMAL INHIBITION OF THE EXCITATORY SYMPATHETIC NERVE RESPONSE TO STIMULATION OF THE FASTIGIAL NUCLEUS IN RABBITS.

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Effects of the cerebellar cortical stimulation on the excitatory responses of the renal sympathetic nerve activity to stimulation of the fastigial nucleus were studied in α-chloralose-urethane (30 and 600 mg/kg, respectively) anaesthetized albino rabbits. The nerve activity was estimated by integrating and averaging impulse discharges after cutting baroreceptor afferent fibers bilaterally. The excitatory responses in the renal sympathetic nerve activity occurred by stimulation of two localized regions of the fastigial nucleus of the cerebellum; one was the rostro-ventro-medial portion and the other the caudo-medial portion. The excitatory sympathetic nerve responses to stimulation of the rostro-ventro-medial portion of the fastigial nucleus was inhibited by preceeding stimulation of cerebellar anterior vermal cortex (lobules II and III), stimulation of which has been known to cause the depression of the ipsilateral renal sympathetic nerve activity (Nisimaru et al., 1977;1979). The extent of inhibition was maximal at about 40 ms (conditioning-testing stimulation interval) and lasted for about 100 ms.

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EFFECT OF KETAMINE ON SYMPATHETIC NERVE ACTIVITY. KUJIME, K. and NINOMIYA I. Dept. of Cardiac Physiology, Research Institute, National Cardiovascular Center, Fujishorodai, Suita, Osaka 565

To investigate the effects of ketamine on the autonomic nervous system, rabbits were anesthetized with pentobarbital and immobilized with pancronium. Renal nerve activity (RNA) and arterial blood pressure were recorded. Six hours after anesthesia, 4 procedures were committed. (1) Changes in RNA in response to intravenous administration of ketamine (5mg/kg) showed initial increase(80%) and subsequent decrease(-25%). (2) Electrical stimulation of the left arotic nerve(.25mA,20Hz) reduced RNA by 84%. Ketamine suppressed this response(-11%). (3) After denervation of bilateral carotid sinus and vago-aortic nerves, ketamine abolished the initial increase and induced more marked subsequent suppression in RNA in comparison with the control. (4) Electrical stimulation of the posterior hypothalamus(.25mA,20Hz) increased RNA to 350% in control. During intermittent stimulation, ketamine induced two different effects, i.e., reduction of the increase to 98% and opposite response to RNA(-11%). From these results, it is concluded that ketamine exerts an inhibitory effect on the baroceptor-hypothalamic-sympathetic systems.

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POST-GANGLIONIC SYMPATHETIC EFFERENT DISCHARGES IN MAN. MANO, T., MIYAOKA, T.* YAMAZAKI, Y.** and KITA, K.*** Res. Inst. Environ. Med., Nagoya Univ., Nagoya 464, * Dept. of Psychology, Hamamatsu Univ. Sch. of Med., Hamamatsu 431-31, ** Nagoya Inst. Technol., Nagoya 466 and *** Dept. of Neurology, Chiba Univ. Sch. of Med., Chiba 280

Post-ganglionic sympathetic efferent discharges were recorded using a tungsten microelectrode inserted percutaneously into the median or the tibial nerve of healthy human subjects. 1. Muscle nerve sympathetic efferent discharges presented spontaneous rhythmic burst activities synchronously with the pulse, modulated by the respiratory cycle, being activated during the inspiration and depressed during the expiration. They were remarkably activated by Valsalva's maneuver, followed by transient depression. They were also activated by head-up tilting of the body. These activations of muscle nerve sympathetic discharges seem to be induced by vasomotor efferent responses to compensate the blood pressure fall. 2. Skin nerve sympathetic efferent discharges showed irregular spontaneous burst activities followed by galvanic skin responses in the palm or the planta. They increased during sensory stimulations and mental activations and were provoked by electrical stimulation of the ipsi-, as well as contralateral nerve trunk with the almost constant latency. These skin nerve sympathetic discharges seem to be dominated by sudomotor efferent responses. The electrically-induced sudomotor response may be provoked by the somatosensory afferent/sympathetic efferent reflex mechanism.

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THE ORIGIN AND PATHWAY OF AUTONOMIC NERVE FIBERS IN THE INFERIOR ALVEOLAR NERVE IN RABBITS. MORITA,M.,IIZUKA,M. and YAMAZAKI,S. Dept. of Physiology, Tokyo Dental College,Chiba City, Chiba 260

Origin and pathway of efferent nerve fibers in the inferior alveolar nerve were investigated in rabbits. The efferent nerve fibers were considered to be sympathetic and parasympathetic because of their conduction velocities and origins. Parasympathe -tic nerve fibers emanated not only from trigeminal nerve but also from glossopharyngeal nerve in the result of denervation. Cutting of the sensory root and/or motor root of trigeminal nerve revealed that the parasympathetic nerve fibers in the trigeminal nerve were located in the sensory root. Parasympathetic nerves from glosso-pharyngeal nerve were present mainly in the mental branch of the inferior alveolar nerve, and the others from trigeminal nerve were observed in the incisal and the mental branch. Of the mental nerve filaments which permit recording of efferent discharges were local-ized in this pathway. One of them innervated a salivary gland minor.

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THE OUTFLOW OF THE AUTONOMIC NERVES INNERVATING THE SUBMAXILLARY GLANDS DURING VOMITING IN DOGS.
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Retching activity (R) was produced by apomorphine injection or intragastric infusions of $CuSO_4$ solutions in chloralose anesthetized dogs immobilized with Flaxedil. 1) Fine nerve filaments dissected from the chorda lingual nerve (CLN) showed a low frequency outflow which was increased markedly before occurrence of R, and then it was interrupted by an inhibition during R. Secretion from the submaxillary glands (SMG) changed parallel to the nerve activity. 2) Sympathetic nerve activity to SMG enhanced before R and followed by a partial inhibition during R. 3) Thoracic esophageal and gastric distentions increased the outflow of CLN and secretion from SMG. Such distentions occasionally elicited R in which they were decreased. 4) Secretion following $CuSO_4$ infusions and esophageal and gastric distentions was abolished after bilateral cervical vagotomy or cutting CLN, but did not decrease after cervical sympathectomy. Grouping discharges in CLN and the fluctuated secretion with a respiratory rhythm were observed after cervical vagotomy. 5) In some cases, the outflow of CLN and secretion from SMG increased periodically with a rhythm of strong gastric antral contractions which occured before and after retching volleys.

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STUDIES ON AFFERENT HEPATIC VAGAL GLUCOSE SIGNAL AFFECTING MOTILITY OF RAT STOMACH.
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Intragastric pressures were recorded in bilaterally adrenalectomized rats with insulin-hypoglycaemia. A significant depression in the pressure was noted following the administration of D-glucose injected into the hepatic portal vein but not after the administration of D-mannose, D-fructose, D-galactose, D-xylose and D-arabinose. The response to the glucose was completely blocked by prior section of the hepatic vagus nerve. Moreover, β-D-glucose was most potent in reducing the pressure in the three forms of D-glucose (α-D-glucose, equilibrated D-glucose consisting of 36% α-D-glucose and 64% β-D-glucose and β-D-glucose). Results suggest that afferent hepatic signals specifically monitoring glucose level in the portal areas vagally modulate gastric motility through the central nervous system and the activation of the hepatic signal is dependent on the anomeric stereospecificity of D-glucose.

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AN ELECTROPHYSIOLOGICAL STUDY OF THE VAGAL PATHWAYS TO THE VISCERAL ORGANS.
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A pair of silver wire stimulation electrodes were placed on the peripheral stump of left or right cervical vagus.

(1) Stimulation of cervical vagus evoked compound action potential of C-wave with or without B response in the abdominal vagus.

(2) Observation of B response indicates the existence of B-fibers:
Ventral and dorsal gastric branch +++, in few cases -
Hepatic branch -, sometimes ±
Ventral and dorsal coeliac branch -, in few cases ±

(3) Left cervical vagus mainly sends fibers to the hepatic branch, ventral gastric branch and ventral coeliac branch through ventral subdiaphragmatic vagal trunk. Right cervical vagus mainly sends fibers to the dorsal gastric branch and dorsal coeliac branch through dorsal subdiaphragmatic vagal trunk.

(4) It seems to be few cross-over vagal fibers between left and right cervical vagi sending fibers to the visceral organs.

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GASTRIC ACID SECRETION INDUCED WITH 2-DG IN CONSCIOUS VAGOTOMIZED DOGS. FUJII,K.,*UEDA, T. AND *TAKASUGI,S. Dept. of Physiology and*Surgery, Hiroshima University Medical Sch., Minami-ku, Hiroshima 734

Influence of truncal gastric vagotomy (TV), splanchnicectomy, administration of atropine and cepharanthine (CR) was studied in conscious dogs on gastric acid secretion from a gastric fistura and denervated corpus pouch on intravenous injection of 2-Deoxy-D-glucose (2-DG).

1. Augmented gastric acid secretion from a gastric fistura on intravenous injection of 2-DG (100 mg/kg/10 min) was greatry inhibited by TV. However, even in TV dogs, 2-DG augmented significantly gastric acid secretion. This esponse disappeard after atropinization (0.05 mg/kg,i.v.).
2. Bilateral splanchnicectomy abolished the augmented gastric acid secretion by 2-DG in TV dogs.
3. Intravenous injection of 2-DG also augmented gastric acid secretion from the denervated corpus pouch of a TVdogs. This response was abolished by CR i.v..

These findings suggest the presence of a neural and neuro-humoral facilitatory mechanism for gastric acid secretion via splanchnic nerve.

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PERIPHERAL MECHANISM OF DESCENDING INHIBITION FROM PONTINE RETICULAR FORMATION ONTO SYMPATHETICALLY MEDIATED INHIBITION OF THE RECTO-RECTAL REFLEX.
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1)The recto-rectal reflex was not changed by guanethidine (Gua., 5 mg/kg i.v.) or 4-7-exo-methylene-hexahydroisoindoline-ethyl-guanidine (No.865-123, 5 mg/kg i.v.) which is an adrenergic blocker with less local anesthetic action. This reflex contraction was not affected by T 13 transection. This result coincides with that of lumbar sympathectomy. 2)Stimulation at sites in the lateral reticular formation evoked sympathetically mediated rectal contraction in animals with cord transection at L 4. This contraction was abolished by atropine (Atr., 0.1-1 mg/kg i.v.) and/or Gua. (3-5). Stimulation at such sites evoked a decrease in lumbar colonic efferent discharges (LCED), which was not affected by Atr. and/or Gua. or No.865-123. 3)Pelvic afferent stimulation (PAS) evoked sympathetically mediated rectal contraction and decreased the LCED via a supraspinal mechanism even after bilateral section of pelvic nerves. Rectal contraction by PAS disappeared after Atr. or Gua. The effect of the LCED was not affected by Atr. and/or Gua. or No.865-123 in the same dosage. These results suggest that the peripheral mechanism involves adrenergic inhibitory neurones which depress activity of cholinergic excitatory neurones in the myenteric plexus.

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REPETITIVE STRAINING ELICITED REFLEXLY BY AFFERENTS FROM THE PELVIC VISCERA OF DOGS. FUKAI, K. and FUKUDA, H. Dept. of Physiology, Kawasaki Medical School, Kurashiki 701-01

Dogs strain repeatedly at stool, as man. It was found in this experiment that the repetitive straining could be induced by distention of the rectum, urinary bladder and vagina in conscious dogs as well as decerebrated dogs. This shows that the straining is elicited reflexly via the neuraxis. Intra-thracheal and -abdominal pressures were simultaneously increased at the reflex straining. Efferent discharges of the phrenic nerve and the nerve to the rectus abdominis muscle were first increased tonically (tonic straining) by these distentions, then the repetitive straining appeared in which both the efferent discharges further increased simultaneously. The repetitive straining was also elicited by afferent stimuli of the pelvic nerve, but not by that of the lumbar colonic and hypogastric nerves which caused only the tonic straining. Lesions were inflicted on the neuraxis to clarify the brain parts composing the reflex arc. The results show that the reflex center of the repetitive straining is located in the ventrolateral part of the rostral pons, and that of the tonic straining exists in the level of the obex.

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VESICO-INTERNAL ANAL SPHINCTER REFLEX. NEYA, T., YAMASATO, T. and NAKAYAMA, S. Dept. of Physiology, Okayama University Medical School, Okayama 700

Vesico-internal anal sphincter(IAS) reflex was studied in cats anesthetized with pentobarbital sodium(30mg/kg,i.p.). Vesical distension or contraction caused tonic contraction of IAS in most animals, but transient relaxation in a few animals. An excitatory response was abolished after section of lumbar colonic(LCN) and hypogastric(HGN) nerves or dorsal roots between T13 and L5, while it was induced after section of pelvic nerve(PN) or spinal transection at lower thoracic segment(LTS). The efferent discharges of anal branch of LCN and HGN increased by vesical distension in most cases. This increase in discharge was resistant to section of PN or HGN, but disappeared after section of both PN and HGN. It did not alter after transection of LTS. The inhibitory response of IAS accompanying an inhibition of LCN efferent discharges was abolished by section of PN or transection of LTS. An inhibition of LCN efferent discharges was completely abolished after transection of LTS. The efferent discharges of anal branch of PN did not alter apparently or decreased by vesical distension.

It is concluded that micturition reflex induced IAS contraction by activation of HGN and LCN efferents which was spinally mediated by HGN and PN afferents and IAS relaxation by inhibition of HGN and LCN efferents which was supraspinally mediated by PN afferents.

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EFFECTS OF URETHRAL AND RECTAL STIMULATION UPON THE BLADDER PRESSURE OF THE ANESTHETIZED CAT. M. MIYATA, K. KURODA* AND S. MORI* Dept. of Urology, Asahikawa Med. Coll., Asahikawa ; Dept. of Physiology, Asahikawa Med. Coll., Asahikawa*

Anesthetized cats exhibit several modes of spontaneous bladder contractions in relation to the intravesical pressure. Attempts have been made to study how such bladder contractions are elicited and what kind of effects can be elicited on them by stimulating the distal urethral and the rectal wall. Bladder was cannulated via a proximal urethra for recording intravesical pressure and for changing intravesical volume. One of the vesical branches of a pelvic nerve was cut to record efferent and afferent nerve discharges. Balloons were placed into the distal urethra and into the rectum for their mechanical distension. When the baseline bladder pressure was between 5 and 20 cm H_2O, periodic bladder contractions with pressure amplitude of 5 to 40 cm H_2O were observed. Preceding a rise of bladder pressure for about 4 s, efferent nerve discharge started to increase. Afferent nerve discharge increased in relation to the increase of bladder pressure. Distension of the distal urethra at low intravesical pressure (below about 40 cm H_2O) increased efferent nerve discharges and intravesical pressure. At high intravesical pressure (above about 40 cm H_2O) the same distension decreased both efferent nerve discharge and intravesical pressure. Distension of the rectum decreased efferent nerve discharge and intravesical pressure with no relation to the level of intravisical pressure.

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SYNAPTIC PLASTICITY IN SYMPATHETIC GANGLIA: I. LONG-TERM POTENTIATION INDUCED BY PRESYNAPTIC ACTIVITIES. KOYANO, K., KUBA, K., KUMAMOTO, E., MINOTA, S. AND NOHMI, M. Dept. Physiol., Saga Medical School, Nabeshima, Saga 840-01.

Plastic increases in the neurotransmitter release after conditioning presynaptic stimulations were studied in bullfrog sympathetic ganglia. The amplitude of the fast excitatory postsynaptic potential (fast e.p.s.p.) recorded intracellularly in a low Ca^{2+},high Mg^{2+} solution increased markedly during and after a brief tetanus (33 Hz, 10 s) to the preganglionic nerves. The increase after tetanus consisted of two components. The fast component lasting several minutes would correspond to the 'potentiation' at the motor nerve terminals, while the slow component lasting 20 min to more than 2 hours would be equivalent to the long-term potentiation in vertebrate central synapses. The slow component was found to be induced by increases in both quantal content and size of the fast e.p.s.p. Thus, the increase in the former was termed presynaptic long-term potentiation (PRE-LTP). The PRE-LTP showed an additive nature to repeated applications of a tetanus, depended on the quantal content before tetanus and on the Ca^{2+} concentration during tetanus, and was not inhibited by lowering temperature. These results may suggest that PRE-LTP is not induced by an energy-dependent mechanism, but presumably by a plastic alteration of the intracellular Ca^{2+} buffering mechanism in the presynaptic nerve terminals.

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SYNAPTIC PLASTICITY IN SYMPATHETIC GANGLIA: II. POSTSYNAPTIC LONG-TERM POTENTIATION.
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In bullfrog sympathetic ganglia, the amplitude of fast excitatory postsynaptic potential (fast e.p.s.p.) recorded in a low Ca^{2+}-high Mg^{2+} solution was increased over 3 h after repetitive antidromic stimulations (20 Hz, 5 s) in a normal Ringer solution. This use-dependent potentiation was associated with an increased quantal size of fast e.p.s.p. A nicotinic acetylcholine potential by iontophoresis was also enhanced by antidromic stimulations in normal Ca^{2+} Ringer without changes in its peak time and the membrane resistance. Such a postsynaptic long-term potentiation (PS-LTP) could not be observed by the tetanus in a Ca^{2+}-free solution. Two mechanisms are possible for the Ca^{2+}-dependent PS-LTP: (1) An increased intracellular Ca^{2+} per se initiates a mechanism for the PS-LTP. (2) A rise in the Ca^{2+} concentration in soma or axon collaterals (observed in 3 of 11 B-neurones) of B- and/or C-neurones causes release of a kind of neurotransmitters (other than adrenaline) which acts on the soma so as to induce the PS-LTP. This PS-LTP may serve as a mechanism for neuronal plasticity in the peripheral autonomic nervous system.

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SYNAPTIC PLASTICITY IN SYMPATHETIC GANGLIA: III. LONG-TERM POTENTIATION INDUCED BY ADRENALINE.
KUBA, K., KUMAMOTO, E., MINOTA, S., NOHMI, M. and KOYANO, K. Dept. physiology, Saga Medical School, Nabeshima, Saga 840-01

The mechanism was further studied of the long-term potentiation of transmitter release by the cyclic AMP-mediated action of adrenaline by recording fast excitatory postsynaptic potentials (fast e.p.s.p.) intracellularly and calculating their quantal content in bullfrog sympathetic ganglia. The lowest concentration of adrenaline to increase the quantal content of fast e.p.s.p. for a long time after its wash (adrenaline-induced long-term potentiation: ADR-LTP) was less than 1 uM, while that to depress the quantal content during its presence was between 1 and 10 uM. The magnitude of the inhibition was not affected by the existence of ADR-LTP, indicating the lack of interactions of two opposing actions of adrenaline. Lowering temperature suppressed ADR-LTP, while not the inhibitory action of adrenaline. The magnitude of ADR-LTP was constant over a range of quantal contents (0.2 - 4.5), indicating the lack of dependence on the extracellular Ca^{2+}. These results suggest that the mechanism of ADR-LTP is independent of the inhibitory action of adrenaline and of the LTP of transmitter release induced by presynaptic activities.

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INTRACELLULAR RECORDING FROM RABBIT OTIC GANGLION.
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Action potential was recorded intracellularly from in vitro preperation of rabbit otic ganglion attached with N. petrosus minor as preganglionic nerve, and N. auriculotemporalis, N. lingualis and N. alveolaris inferior as postganglionic nerves. The electrical property of the ganglion cells was examined.

The averages of input resistance input, capacitance and time constant of cell membrane were 30.8±21.9MΩ, 176±101pF and 4.9±3.6 msec, respectively. The average of the conduction velocity of postganglionic fiber was 0.86±0.35m/sec, although that of preganglionic fiber could not be estimated because of very short N. petrosus minor dissected. Many cells showed an afterhyperpolarization following direct and orthodromic spikes, which had a duration of over 400 msec. In one of these cells, the afterhyperpolarization was sometimes followed by a long lasting hyperpolarization over 5 sec in the course of reverting to the resting potential. The repetitive stimulation at over 0.5 Hz resulted in the disappearance of the long lasting hyperpolarization. The detail mechanism of the long lasting hyperpolarization will be analysed in future. In some neurons, slow e.p.s.p.s and slow i.p.s.p.s were recorded by the repetitive stimulation of preganglionic fiber.

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INTRACELLULAR STUDIES ON THE PTERYGOPALATINE GANGLION OF RABBIT. HOSHINO, T., YOSHIZAKI, K., SATO, M. and KOYANO, H. Dept. of Physiol. Akita Univ. Sch.of Med., Akita 010

Experiments were carried out on cells in the pterygopalatine ganglion, together with the vidian nerve (pre-ganglionic nerve) and the post nasal nerves (post-ganglionic nerves) dissected from the animal.

The fast e.p.s.p.s were recorded from the PPG cells in response to stimulation of the vidian nerve and also the post nasal nerve, which were inhibited by d-TC (10^{-5}M). Antidromic action potentials were evoked by stimulating the other post nasal nerve. The experimental results suggest the existence of the ganglionic refelx in the PPG, as in the coeliac ganglion of rabbit. The averages of conduction velocity of pre-ganglionic fibers in the vidian nerve and the post nasal nerves were 1.97±1.21m/sec and 0.998±0.494m/sec. As with post-ganglionic fibers, the average was 2.13±2.75m/sec. The averages of membrane resistance and capacitance, and time constant of the PPG cells were 28.1±18.1MΩ, 162±112pF and 3.90±2.98 msec, respectively. In addition, slow e.p.s.p.s and i.p.s.p.s were also recorded in some neurons, which were inhibited by atropine (10^{-6}M).

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CIRCULATORY RESPONSES EVOKED BY STIMULATION OF THE MOTOR CORTEX AND THE HYPOTHALAMUS. KOGA, T., ISHIKAWA, T., MIYAZAWA, T. Dept. of Physiology, Nihon Univ. Sch. of Med., Itabashi-ku, Tokyo, 173

The neurophysiological properties of the motor cortex and the hypothalamus that activate the sympathetic vasodilator system were studied using vagotomized cats anesthetized with chloralose-urethane. Atropine sensitive vasodilatation and inhibition of carotid sinus baroreflex were observed by stimulation of motor cortex, and simultaneously field potentials were recorded in the amygdala and the hypothalamic defence area especially around the anterior hypothalamus. These potentials were consisted of two negative peaks which had 3-5ms and 20-30ms of latencies respectively. The latter was considered to be a multi-synaptic response because it was decreased or faded with asphyxia, frequent stimulation above 10 Hz and administration of small dose of Nembutal. Furthermore, by stimulation of the region in which evoked potentials were recorded, atropine sensitive vasodilatation, inhibition of baroreflex and change of the renal nerve activity were observed as well as the case of the motor cortex stimulation. It is suggested that the motor cortex is related to interaction with the circulatory reflex through the sympathetic vasodilator system including the anterior hypothalamus.

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EFFECT OF CAPILLARY BLOOD FLOW ON OXYGEN TRANSPORT TO TISSUE.
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In capillary blood flow, plasma shows a different behavior from RBC. In addition, the oxygen dissolved in plasma is not always in equilibrium with RBC oxygen. It is the purpose of this report to develop a mathematical model for describing effects of non-equilibrium and relative motion between RBC and plasma to elucidate microrheological factors of the oxygen transport to tissue. A two-fluids model of RBC and plasma is introduced for blood, including effects of hemoglobin release in RBC and of a variation of capillary hematocrit. For a capillary-tissue unit, a tissue cone model (a modified Krogh model) is devised to describe a capillary pattern with symmetric antiparallel input and output. Equilibrium and non-equilibrium blood flows are studied on the basis of our model for various parameters such as the RBC velocity and the RBC flux. Especially, the oxygen transport to tissue from capillary is examined numerically, using published physiological data. It is found that not only the RBC flux but also the capillary hematocrit becomes important in the oxygen transport to tissue. Especially, hemoglobin release effect affects the non-equilibrium flow under the low hematocrit, which leads to enlarging the region of the lowest oxygen pressure in tissue.

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THE EFFECT OF CAROTID SINUS NERVE STIMULATION ON THE PERIODICITY OF CAPILLARY RED CELL FLOW VELOCITY IN THE RABBIT TENUISSIMUS MUSCLE.
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The microcirculation of the tenuissimus muscle in urethane-anesthetized rabbit was observed micrscopically. The muscle with the main artery and vein was placed on the stage which was composed of two prisms connected to the light source with optic fibers. The magnified image of the transilluminated light by a zoom stereo microscope was projected on TV monitor through a TV camera. The muscle was suffused with Tyrode's salt solution to control pO_2 and pH at desired physiological. The common carotid artery was canulated to monitor arterial pressure and the carotid sinus nerve was isolated at the carotid bifurcation and was stimulated electrically. At the control state the red cell flow velocities at capillaries showed periodic fluctuations, sometimes accompanying the intermittent cessation of flow. When carotid sinus nerve was stimulated(6 - 10 V,100Hz square wave), the periodicity of the flow was markedly reduced or almost disappeared while the mean capillary flow velocity increased maximally 3 to 5 times of the control level.

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EFFECTS OF SYMPATHETIC BLOCKADES AND NOREPINEPHRINE ON INTERNAL DIAMETER OF SMALL ARTERIES IN THE RABBIT HINDLIMB -- ANGIOGRAPHICAL ANALYSIS --
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Neurohumoral control of internal diameters (ID) of the femoral artery (FA) and its branches (profundal and circumflex arteries) was studied with administration of sympathetic ganglion blockade (hexamethonium,i.v.), norepinephrine and α-receptor blockade (phentolamine,i.v.) in rabbits. Serial measurements of ID's of FA and its branches in a length of 100 mm, 50 mm and 30 mm were made on angiograms obtained with an X-ray apparatus, respectively. Sympathetic neural control was seen mainly in the branches. In contrast, humoral control with norepinephrine was seen in both FA and the branches. The relative changes in ID in response to above mentioned procedures were different not only between FA and the branches but also within FA. These results indicate that ID of rabbit hindlimb arteries was nonuniformly and selectively controlled by sympathetic nerve activities and plasma norepinephrine.

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NON-UNIFORM DISTENSIBILITY CHARACTRISTICS IN THE VENOUS SYSTEM : FROM THE MORPHOLOGICAL POINT OF VIEW MURASE, K., OHHASHI, T. and AZUMA, T. Dept. of Physiology, Shinshu University School of Medicine, Matsumoto 390

Pressure-radius and pressure-volume relationships in canine veins were investigated to examine regional differences in their circumferential distensibilities. Excepting the thoracic and suprarenal portions, the distensibility of the caval vein was larger than those of the extremity veins. Among the extremity veins, there existed a general tendency that the more distally the segment was located, the smaller the distensibility became. Morphological observation revealed regional differences in amount and alignment direction of elastic and smooth muscle fibers in the walls of venous segments isolated from various portions. Non-uniform distensibility characteristics in the venous system were considered on the basis of the local differences in architecture of the fibrous components in the walls.

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A CAMERA-TYPE PLANIMETER GAUGE APPLICABLE TO SMALL BLOOD AND LYMPH VESSELS. #SAKAGUCHI, M., OHHASHI, T. and AZUMA, T. #Dept. of Electrical Engineering, Nagano Technical College, Nagano 380, and Dept of Physiology, Shinshu University School of Medicine, Matsumoto 390

We have designed and constructed a planimeter gauge utilizing the area image sensor which has attracted special interest recently as a new photoelectric transducer. The gauge was stable, inexpensive, small in size, and easy to assemble. A combination of this gauge with a monocular reflex camera enabled us to record continuously changes in the projected area of a blood or lymph vessel on the focal plane. This device has the following advantages. (1) Contact-free measurements were possible, avoiding undesirable mechanical influences on the vessel wall. (2) The resolving power of the sensor was sufficiently high, i.e., 4100 μm^2 in principle. (3) The frequency response of the device was flat up to 20 Hz, the frequency range being sufficiently wide to cover usual cardiocascular phenomena.

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SKIN BLOOD FLOW MEASUREMENT BY HEAT FLOW COMPENSATION TECHNIQUE. FUKUOKA, M., SHIMAZU, H., ITO, H., TOYOSHIMA, T.*. TOGAWA, T.* and YAMAKOSHI, K.** Dept. of Physiol., Kyorin Univ. School of Med.; * Tokyo Med. & Dent. Univ.; ** Hokkaido Univ.

A new method to measure the skin blood flow was developed using a heat compensation technique. In this method a compartment model which is thermally insulated from the ambience was assumed in the skin. Between the thermal insulator and the skin a heater plate was placed, and kept the compartment temperature higher than the reference value initially set. Theoretical consideration on the heat balance in the compartment showed that the heater power to keep the temperature constant was approximately proportional to the skin blood flow. Using this method the skin blood flow in the palm was measured, and the values in a range 1 ~5ml/min/100cm² was obtained in the room temperature around 18 ~28℃.

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RELATION BETWEEN PULSE WAVE PROPAGATION TIME AND PERIPHERAL RESISTANCE. OKAI, O., HIGUCHI, Y. and MAKINO, K. Dept. of Physiology, kyorin University School of Health Science, Miyashita 467, Hachioji, Tokyo 192

The first experiment was performed on the rabbit thigh. In the various circulatory conditions induced by intravenous infusion with noradrenaline, isoproterenol and acetylcholine the peripheral resistance of the femoral artery were related more closely to the square root of the pulse wave propagation time (PWPT) than to PWPT (linea relation) and a decrease in hematocrit decreased the proportional constant. The second experiment was carried out on the arm and the neck in man. PWPT were measured as the duration from the upward deflecxion of the brachial arterial flow wave, detected by a ultrasonic flowmeter, at the cubital joint region to the upward deflecxion of pulse wave of the second finger, detected by a impedance plethysmograph. Closer correlation coefficient $r_1 = 0.864$ was observed for the square root equation than the coefficient $r_2 = 0.802$ for the linear equation. Another PWPT was the duration from the upward deflecxion of left carotid arterial flow wave to the upward deflecxion of the pulse wave measured at the left auricle. Closer correlation coefficient $r_3 = 0.990$ was observed for the root square equation than the coefficient $r_4 = 0.883$ for the linear eqation.

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EFFECT OF HEMODYNAMIC PARAMETERS OF THE AORTA ON THE BLOOD VELOCITY PROFILE. TAKEUCHI, A., SATO, T., SHIRATAKA, M., NARA, Y., and IKEDA, N. Depts of Medicine, Physiology, and Information Science, School of Medicine, Kitasato University, Sagamihara, Kanagawa 228

We have previously reported that the aortic blood velocity profile with the " reflection wave" and "after vibration" are changed greatly by administration of vasodilators such as sodium nitroprusside and trinitroglycerole. (J. Physiol. Soc. Japan 44 (8,9), 1982) It was then suggested further by the simulation studies that the parameters that affect the blood velocity profile are proximal capacitance and resistance rather than the peripheral resistance contrary to the general idea about the terminal impedance. The present report deals with an experimental study to elucidate the mechanism that underlies the vibrating blood velocity pattern. Either Norepinephrine 0.1 mg, etilefrine hydrochrolide 2 mg or 5 mg, or glass beads of 100 μm diameter were injected into the aortic arch of the mongrel dogs under nembutal anesthesia and aortic flow rates and pressures were measured. Frequency of the after vibration was increased by any of the above treatments whereas the amplitude did not vary consistently. The vasodilators seemed to slow the vibration frequency so that the after vibration could have been hidden by the succeeding ejection waves. Capacitances of the arteries may be a primary factor of the "after vibration".

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RELATION WITH LEFT VENTRICULAR EJECTION FRUCTION AND CARDIAC OUTPUT IN HYPERTROPHIC AND DILATED HEART. FURUKAWA,Y., SHIMIZU,M.,MAKITA,K., TOMIYA,H.,MIZUNO,T., SUGUYAMA,Y., SAITO,T. and INAGAKI,Y. The Third Dept. of Internal Med., Chiba Univ. Sch. of Med., Chiba 280

We studied in hemodynamic parameters of hypertrophic and dilated heart in 34 patients with essential hypertension and 47 patients with myocardial infarction, comparing them with a control group of 13 normal cases. And we also studied the response to exercise testing in patients with essential hypertension.

The results were as follows-

1) Cardiac index and stroke index were not different in hypertrophic or slightly dilated groups as conpared with normal group.
2) In myocardial infarction patients, ejection fraction had a tendency to decrease as CTR increased, but cardiac index and stroke index maintained normal levels in which CTR was under 60%.
3) In the exercise test of hypertension group, patients with hypertrophy or with over 50% of CTR had a tendency in which ejection fraction was decreased by exercise as compared with patients without hypertrophy or not having over 50% of CTR.

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DIRECT RELATIONSHIP BETWEEN BLOOD PRESSURE AND VISCOSITY IN PATIENTS WITH ESSENTIAL HYPERTENSION. NAKAMURA, Y., HIGAKI, S., FUKUDA, T. and UDA, T. Dept.Med. Yohkaichiba City Hospital, Yohkaichiba Chiba 289-21

The direct effects of blood fluidity on blood pressure were studied in 80 patients with essential hypertension, whose diastolic blood pressure was over 95 mm Hg. Whole blood viscosity at the shear rate of 230 sec-1 correlated significantly with blood pressure ($r=0.44$, $p < 0.01$). Whole blood viscosity values measured at 5 different shear rate, 11.5 to 230 sec-1, in high renin hypertension were significantly different from those in control, low or normal renin hypertension ($p < 0.02$ to 0.001). Viscosities of 40(female) or 45(male) % red blood cell(RBC) in 5 % albumin-Ringer solution (shear rate, 230 sec-1) and that of 40 or 45 % RBC in autologous plasma (shera rate, 11.5 sec-1) increased significantly($p < 0.02$ to 0.001) and RBC filtrability through 5 u pores decreased significantly ($p < 0.05$ to 0.02) in high renin hypertension compared with those in normal control and low renin hypertention. Such data suggested decrease in RBC deformability and increase in RBC aggregability, especially in venules,in high renin hypertension.

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PLASMA LIPIDS AND CUTANEOUS MICROCIRCULATION IN EARLIER STAGE OF DIETARY INDUCED ATHEROSCLEROSIS IN THE RABBIT. ASANO, M., HIROKAWA, A., OHKUBO, C., OGAWA, S., SASAKI, A., SAWANOBORI, K., YONEKAWA, K., and YOTSUYA, T. Dept. of Physiological Hygiene, the Institute of Public Health, Minato-ku, Tokyo 108

In normal male rabbits during cholesterol feeding(1.5% cholesterol supplemented diet) for a period of 4 weeks, marked increase in plasma levels of total cholesterol, LDL-cholesterol and triglyceride and in atherogenic index(LDL-cholesterol/HDL-cholesterol, A.I.) developed progressively. Concurrently with the plasma lipid increment, marked elevation of intravascular adhesiveness of circulating leukocytes and abnormal intravascular aggregation of flowing erythrocytes were observed vital-microscopically in the cutaneous microcirculatory system within a transparent chamber installed into the ear lobe. During the same period, loading of whole phase or gas phase of cigarette smoke was performed two times a day but any appreciable changes in the plasma lipid levels and the intravascular behaviors of leukocytes and erythrocytes were not observed throughout. During combined loading of dietary cholesterol and whole phase or gas phase of smoke, less pronounced increase in the plasma lipid levels developed, and less increase in A.I. developed only for the latter combination. Microcirculatory changes were observed to be more or less enhanced during each combined loading of cholesterol and whole and gas phase of cigarette smoke.

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THE STUDY OF THE PHYSICAL PROPERTIES OF THE AORTIC WALL IN THE WHITE RABBIT FED A HIGH CHOLESTEROL DIET. MIYAZAKI,A., FUKUDA,K.*, HONDA,M.*, NISHIMOTO,Y.*, TAKAHASHI,M.*, YAMAZAKI,S., SHUKUYA,M., MASUDA,Y., and INAGAKI,Y. The Third Department of Internal Medicine, Chiba University School of Medicine, Chiba, 280

For analysis of the physical properties of the atherosclerotic aortic wall, 39 male white rabbits were divided in the two groups, one group(n=19) fed an atherogenic diet containing 1% cholesterol (group A), and the other(n=20) fed a control diet (group B). The feeding period was from 8 to 20 weeks. Average aortic pulse wave velocity at a diastolic blood pressure of 100 mmHg was greater in the group A than in the group B. The aortic wall thickness of the group A increased due to the development of the atherom within intima and inner portion of media. The breaking strength and the circumferential static modulus of elasticity (Young's modules) were less in the group A than in the group B. But the product of the Young's modulus and the wall thickness was more increased in the group A rather than in the group B. Therefore the increased stiffness of the atherosclerotic aorta in this study appeared to be due to the increased wall thickness mainly rather than the Young's modulus.

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ROLE OF CARDIAC SYMPATHETIC NERVES FOR THE PRODUCTION OF A SYSTEMIC ARTERIAL PRESSURE OSCILLATION. HAYASHI, M. and MIYAKAWA, K. Dept. of Physiol., Shinshu Univ. Sch. Med., Matsumoto 390

The blood supply to the brain was surgically limited to one common carotid artery in anesthetized rabbits. When the common carotid was compressed by exertion of side pressure, the level of systemic arterial pressure (SAP) rose and SAP oscillation appeared. Respiratory movement, ascending aortic flow, heart rate and SAP were simultaneously recorded. During the SAP oscillation, the cardiac sympathetic nerves were cut under condition with the buffer nerves intact. In 3 cases of 8 rabbits, SAP oscillation disappeared by cutting bilaterally the cardiac sympathetic nerves. In 5 cases SAP oscillation continued even after it. When the oscillation appeared, the level of SAP rose to 106 ± 6 mmHg (Mean ± SE) from 65 ± 5 mmHg before exertion of side pressure in the former cases, and to 121 ± 6 mmHg from 67 ± 6 mmHg in the latter. The ratio of the duration of descending phase of the SAP oscillation to that of ascending phase decreased by cutting the cardiac sympathetic nerves. It was 1.25 ± 0.04 and 1.08 ± 0.06 before and after the denervations respectively in the former cases and also 1.51 ± 0.07 and 1.40 ± 0.06 in the latter. The results indicate that the cardiac sympathetic nerves actively contribute to the production of SAP oscillation, especially to the formation of the ascending phase of it.

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VASOMOTION CHANGE EVOKED BY PERIPHERAL BLOOD FLOW INHIBITION. UEDA,G., TAKEOKA,M., KOSHIHARA,Y. and SAKAI,A. Dept.of Adapt. Physiol., Shinshu Univ. School of Medicine, Asahi, Matsumoto, 390

When some side pressure was applied to the carotid artery of a rabbit, blood pressure oscillations take place. Similarly, when the central artery of the earlobe was partially occluded, the distal or proximal portions near the constriction begin to dilate or constrict alternately after some latency. On the contrary, however, the oscillatory change sometimes disappear by the procedure of occlusion. This phenomenon of evoking is seen in less than a half of the total experiments. Not the 42 ℃ pretreatment, 20 min, but 15 ℃ pretreatment seemed to cause larger oscillations after the occlusion. After the release of the occlusion, a kind of reactive hyperemia superimposed by conspicuous oscillations could be noticed sometimes. The type of oscillation is not so regular, compared with the cases in cold stimulus of -7 ℃. These changes were recorded by thermistor pickups placed on the vessel. When the laser-Doppler method of flow measurement (Canon,PERIFLUX) was attempted, significant changes of flow were noticed mostly corresponding to the temperature recording.

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Rabbit's baroreceptor reflexes and arterial pressure restoration after hemorrhage. Ishikawa, N. and Sagawa, K.* Dept. of Pharmacology, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan and Dept. of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, U.S.A.

To assess the interaction among the baroreceptor reflexes, 6 rabbits were bled under the following conditions; 1) with all intact baroreflexes, 2) without aortic arch reflex, 3) without carotid sinus reflex and 4) without aortic arch and carotid sinus reflexes. In other 6 rabbits, the order was changed; elimination of carotid sinus reflex was the second and elimination of aortic arch reflex was the third. And in the latter group, elimination of vagus nerves was added; 5) without aortic arch and vagal mediated cardiopulmonary reflexes, 6) without all reflexes. Under all these conditions, blood, 4.5 ml/Kg B.W., was withdrawn from the femoral artery. From the results of arterial pressure restoration in the hemorrhagic disturbance, the gain of each baroreceptor reflex was calculated under the linear assumption for the reflex characteristics. Gain for the vagal function was near to zero, 0.26 ± 0.21. The gain values of carotid sinus and aortic arch baroreflexes were 2.37 ± 0.40 and 2.88 ± 1.03, respectively. The gain value for the reflex loop of the interaction among the baroreceptor reflexes was 2.69 ± 0.86. Furthermore, the effect of hemorrhage on the open-loop characteristics of carotid sinus baroreflex was also studied.

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LOCAL AND REFLEXOGENIC REGULATION OF RENAL BLOOD FLOW IN DOGS.

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The local and reflexogenic effects of a quick mild hemorrhage (2ml/l-2sec/Kg body weight) on the renal vascular resistance (RVR) were studied on 23 dogs anesthetized by pentobarbital sodium (30mg/Kg b.w. i.v.). The hemorrhage was carried out by piston-syringe system connected to a large bore catheter inserted into the aortic arch. Renal arterial pressure (RAP) was monitored by a catheter-transducer system inserted into the abdominal aorta. An electromagnetic flowmeter probe was placed around the renal artery. RAP, renal blood flow (RBF) and RVR calculated by dividing RAP by RBF were recorded on a strip chart. The hemorrhage experiment was repeated several times under nerve intact condition and after sectioning the carotid sinus and the vagus nerves bilaterally. We measured control RBF (CRBF), control RVR (CRVR) before the hemorrhage and maximum change of RVR (ΔRVR) after the hemorrhage. ΔRVR increased with increasing CRBF. ΔRVR's under the denervated condition were always negative. We conclude that the renal vessels under the baroreceptors denervated condition were dilated by the local regulation of RBF. The renal vessels under the nerve intact condition were dilated if CRBF was smaller than 3 ml/min·g and were constricted if CRBF was larger than 4 ml/min·g.

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REFLEX GAINS OF THE BAROREFLEX AND THEIR INTERACTING SYSTEMS IN THE RABBIT.

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The goal of this study was to determine the reflex gains (G) of the carotid sinus (CS), aortic arch (Ao), cardiopulmonary (Cp) and their interacting baroreflex systems in the rabbit. The reflex gain was determined from arterial pressure response to a quick mild hemorrhage (2ml/l-2sec/kg body weight) in 81 anesthetized Japanese white rabbits. G was repeatedly determined under several nerve conditions, i.e., nerve intact condition (INT), after sectioning the CS-nerves ($\overline{CS}$), the Ao-nerves ($\overline{Ao}$) or the vagi ($\overline{V}$) and any two of them, denoted as $\overline{CS+Ao}$, $\overline{Ao+V}$ and $\overline{V+CS}$. Finally, we confirmed that G became zero after sectioning all of the buffer nerves ($\overline{CS+Ao+V}$). G(INT) was 7.4. G($\overline{CS}$), G($\overline{Ao}$) and G($\overline{V}$) were 3.0, 2.8, and 3.8. G($\overline{Ao+V}$), G($\overline{V+CS}$) and G($\overline{CS+Ao}$) mean G(CS), G(Ao) and G(Cp), and were 1.8, 1.6 and 0.9, respectively. G(CS*Ao), G of the interacting system between CS- and Ao-systems, was estimated to be 0.4 by subtracting G(CS) and G(Ao) from G($\overline{V}$). Similarly, G(Ao*Cp) and G(Cp*CS) were 0.5 and 0.1. G(CS*Ao*Cp), G of the interacting system among CS-, Ao- and Cp-systems, was estimated to be 2.1 by subtracting G(CS), G(Ao), G(Cp), G(CS*Ao), G(Ao*Cp) and G(Cp*CS) from G(INT).

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CHANGES OF ARTERIAL Po_2 AND Pco_2 BY QUICK MILD HEMORRHAGE.

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Open-loop gain of the arterial pressure control system is usually higher in value in compared to those reported by others, when it is estimated by the method of the quick mild hemorrhage. The reason may be that the hemorrhage might change not only baroreceptor but also chemoreceptor activities. In this study, recordings of arterial Po_2 and Pco_2 were carried out during the quick hemorrhage in order to answer the question. Mongrel dogs under pentobarbitone anesthesia (30 mg/Kg b.w.) were used. Measurements of the arterial Po_2 and Pco_2 were done using sensors for each gas placed within both common carotid arteries. Arterial Po_2 increased by more than 6 mmHg immediately after the hemorrhage, whereas arterial Pco_2 decreased by more than 1 mmHg in certain time delay after the hemorrhage. The changes in the arterial Po_2 and Pco_2 during the hemorrhage could not be observed in the animal with the baroreceptors denervated conditions, or when the respiratory movement was kept constant using the artificial respiration. Thus, increase in Po_2 and decrease in Pco_2 are due to hyperpnea induced by altered baroreceptor activity through the hemorrhage, suggesting that the arterial pressure fall caused by the hemorrhage is the input to the rapidly acting pressure control system.

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A POSSIBLE FUNCTION OF THE GLOMUS CELL IN CONTROLLING BLOOD FLOW OF THE CAROTID LABYRINTH IN XENOPUS LAEVIS. KUSAKABE, T., ISHII, K. and ISHII, K. Dept. of physiology, Fukushima Medical College, Fukushima 960

The carotid labyrinth of Xenopus laevis has a chemoreceptor function and contains glomus cells similar in fine structure to those of other animal species. Electrone microscopic study showed that some of the glomus cells connected intimately with smooth muscles in the labyrinth (g-s-connection). Efferent electrical stimulation of the glossopharyngeal nerve resulted in a concentration of dense-cored vesicles in the peripheral site of the cell, reducing the number as a whole, and facilitating exocytosis at the g-s-connection. In an artificially perfused carotid labyrinth, outflows of the internal and external carotid arteries decreased with administration of catecholamines. NaCN and Ach reduced only the internal outflow. Phentolamine depressed the effect of NaCN. Response to Ach was depressed by Hexamethonium and Phentolamine. These results suggest that the glomus cell may participate in controlling blood flow in the labyrinth through the intervention of g-s-connection.

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EFFECTS OF PHYSIOLOGICAL VASOACTIVE SUBSTANCES AND GASTROINTESTINAL HORMONES ON SPONTANEOUS MECHANICAL ACTIVITY IN THE CANINE PORTAL VEIN. NAKAGAWA, H., OHHASHI, T., and AZUMA, T. Dept. of Physiology and Research Lab. for Cardiovascular Disease, Shinshu University School of Medicine, Matsumoto 390

The present study was undertaken to elucidate the physiological significance and the mode of humoral regulation of spontaneous mechanical activity in the canine portal vein. The spontaneous contractions of isolated portal preparations originated in longitudinal smooth muscle layers which were well-developed in the wall of the portal trunk near the liver. Some vasoactive substances and gastrointestinal hormones affected the rhythmicity and amplitude of the spontaneous contractions. Increases in the rate of contraction were induced by 5-hydroxytryptamine (5-HT), norepinephrine (NE), epinephrine (Ep), acetylcholine (ACh), prostaglandin $F_{2\alpha}$, histamine and ATP. The pacemaker cells were particularly sensitive to 5-HT. The effects induced by Ep and NE were competitively blocked by phentolamine. Increases in contraction rate produced by ACh and histamine were inhibited by atropine and diphenhydramine, respectively. Secretin caused a dose-related increase in the rhythm, in sharp contrast to insulin which brought about a dose-dependent decrease. The former effect was unaffected by phentolamine and the latter by propranolol.

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RESPONSE OF EXTRACELLULAR SPACE TO SALINE LOADING IN RAT. YAMADA, S., NOSE, H., MORIMOTO, T. and OGURA, K. Dept. of Physiology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602.

To quantitate the distribution of saline administered intravenously (2 ml, 3 ml, 4 ml/100 g of body weight) among vascular space, interstitial fluid space (ISF) and lymph, blood volume was continuously monitored and thoracic duct lymph was collected simultaneously using rat as experimental animal. Ratio of fluid shift from vascular space into extravascular space was constant irrespective of the infused volume, i.e., 70% at the end of infusion and 90% at 50 min after the end of infusion. However, the rate of lymph flow through the thoracic duct varied depending on the volume infused, and amounted to 6%, 11%, and 18% of the infused volume in the group with 2 ml, 3 ml and 4 ml of infusion, respectively. More than 70% of the saline was imbibed into interstitial fluid space. Based on the result, the characteristics of the lymph production were analyzed using simulation analysis. The resistance against water flow from ISF to lymph was about a half of that through vascular wall. The hinderance against protein transport was also found. These finding suggest the buffering capacity of ISF for water to maintain blood volume constant.

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PRE- AND POSTJUNCTIONAL ALPHA ADRENOCEPTORS IN BOVINE MESENTERIC LYMPHATICS. OHHASHI, T., and AZUMA, T. Dept. of Physiology, Shinshu University School of Medicine, Matsumoto 390

We studied isolated bovine mesenteric lymphatics to investigate the pharmacological properties of pre- and postjunctional alpha adrenoceptors. Rings of the lymph vessels were mounted for isometric tension recording in an organ bath filled with Krebs-Ringer bicarbonate solution. Phenylephrine and clonidine caused contractions of the preparations dose-dependently. Alpha antagonist, yohimbine was a competitive antagonist against phenylephrine and clonidine in the lymphatic smooth muscles. The Schild's plot for yohimbine in the lymph vessels suggests that the presence of both alpha 1- and alpha 2-like adrenoceptors on the smooth muscle cells. The action of phenoxybenzamine, yohimbine, noradrenaline and clonidine on ^{3}H-noradrenaline release induced by nerve stimulation was also studied in the lymphatic preparations. The former aplha-antagonists increased the ^{3}H-noradrenaline release, and the latter alpha-agonists decreased. These findings suggest that there exist the prejunctional alpha-autoreceptors which regulate the release of noradrenaline from nerve terminals through a negative feedback mechanism.

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Po_2 IN TENDON AND THE SURROUNDING TISSUES OF THE RABBIT HINDLIMBS. TAKEMIYA,T.,MAEDA,J.,HAGIHIRA,M.and KANEYUKU,T. Div.of Physiol., Inst.of Health & Sports Sci., Univ.of Tsukuba. Sakura, Niihari, Ibaraki 305

With spontaneously stabilizing arterial blood pressure after urethane anesthesia, continuous measurement of Po_2 was performed by polarographic method using a wire type of platinum electrode covered with a polymer membrane. The size of electrode was 80 μm in diameter, which was introduced into the tissues of tibialis anterior by the aid of fine needle after the check of standard calibration. The amounts of tissue Po_2 at rest (mmHg,mean±SE,n) were in order of Muscle(64.6±16.2,39), Tendon Surrounding (44.2±7.5,30) and Tendon(30.4±14.9,61). The data in tendon depended much on the local geography. After the cessation of pure oxygen inhalation,the decreasing rate of tissue Po_2 was larger in the tendon surrounding than the tendon tissue. The effect of sciatic nerve cutting showed a gradual increase of Po_2 in all tissues used. In muscle exercise induced by electrical stimulation of sciatic nerve end after cutting, 5 Hz intensity produced a marked decrease of Po_2 in muscle, but a slight in tendon, and no change in tendon surrounding. These data suggest that the changes of Po_2 in tendon tissue depend on the alternative activity of microvascular segmental blood supply system in these tissues.

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IS THERE A BLOOD PRESSURE RECEPTOR IN BRAIN? HATAKEYAMA, I., TAKEI, Y. and KATAKURA, T. Department of Physiology, Kitasato University School of Medicine, Sagamihara 228

In rabbit's brain, the communication of blood vessels between the route via internal carotid artery and the route via vertebral artery is so insufficient that circle of Willis is not formed. Therefore the rapid transfusion of rather small amount of blood into internal carotid artery may produce a prompt rise of cerebral blood pressure without significant rise of systemic blood pressure. We have investigated on the effect of the elevation of cerebral blood pressure (CBP) by rapid constant flow transfusion (0.5 ml/s - 1.5 ml/s) of 5 ml blood into internal carotid artery of one side. CBP was measured at interval carotid artery of another side. CBP rised promptly about 5 - 20 mmHg and gradually dropped while systemic blood pressure (SBP) at aortic arch markedly dropped after initial slight rise at most cases. Simultaneously heart rate was lowered and respiration was slightly inhibited. The hypotensive effect, however, disappeared and gradual rise of SBP remained when SBP was considerably lowered by depletion of 10 - 20 ml blood. The rise of SBP is considered to be the effect of increase of blood volume since similar hypertensive effect was produced by blood transfusion into extracranial vessels via external carotid artery by the same manner. It is suggested that a blood pressure receptor, which has higher threshold compared with that of aortic or carotid sinus baroreceptor, exists in brain.

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EVIDENCE FOR CEREBROVASODILATORY EFFECT OF CARDIOVASCULAR REFLEX ARC AS EVIDENCED BY ELECTRICAL STIMULATION OF NUCLEUS TRACTUS SOLITARIUS. NAKAI,M., AND OGINO,K. National Cardiovascular Center Research Institute, Suita, Osaka 565

An issue has yet been settled whether or not the cardiovascular reflex arc regulates the cerebral vasculature. The reports so far available, however, depended on studies which used physiological stimuli for activating the cardiovascular receptors. For this reason we designed present study by a use of electrical stimulation of the intermediate region of the nucleus tractus solitarius (NTS) to unspecifically activate an ascending efferent limb of the cardiovascular reflex arcs. Regional cerebral blood flow was determined by Kety's principle with iodoantipyrine in anesthetized (N_2O), cordotomized (C_2), ventilated 21 rats with the vago-sympathetic trunks cut. Arterial pressure was maintained within physiological range by iv infusion of phenylephrine. Arterial O_2 and CO_2 tensions were also kept physiological. In response to stimulation (50Hz, 0.5msec, 1 sec on/1 sec off, 60 $\pm$ 13 SE uA, n=9), the rostral and caudal cerebral cortices and caudate nucleus significantly (P 0.05) increased their flow up to 169 $\pm$ 17 ml/min/100g (181% of control), 132 $\pm$ 7 (143%), and 109 $\pm$ 8 (125%), respectively. We conclude that some unknown cardiovascular reflex arc dilates the cerebral vessels, whereas its effect is muffled by other vasoconstrictor mechanisms in physiological circumstances.

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EFFECTS OF EXTRACRANIAL OCCLUSION OF CAROTID AND VERTEBRAL ARTERIES ON THE SYSTEMIC CIRCULATION IN RABBITS. SHIMIZU, T. and MIYAKAWA, K. 2nd Dept. of Physiol., Shinshu Univ. Sch. Med., Matsumoto 390

The common carotid, external carotid, internal carotid and vertebral arteries were bilaterally exposed near their origin in anesthetized mature rabbits. They were alternately or cumulatively occluded in various order and combinations. The systemic arterial pressure (SAP) was recorded simultaneously with the heart rate (HR) and respiratory movements. The SAP and HR showed no change by vertebral occlusion in any experimental condition. Common carotid occlusion produced typical baroreflexes which continued as long as the common carotid was clipped even after both vertebral occlusion. When one external carotid was occluded following occlusion of contralateral common carotid and both vertebrals, the SAP rose slightly without change of the HR, but it did not change when the ipsilateral internal carotid was occluded instead of the external carotid. These results are interpreted as that the blood supply to the brain through only one route among six arteries of bilateral internal carotid, external carotid and vertebral is sufficient for maintaining the brain function relating to circulatory regulation. This interpretation is also supported by our previous study, which demonstrated angiographycally an observation that each of common carotid and vertebral arteries had a capacity to supply blood to both sides of the brain by itself.

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HEMODYNAMIC CHARACTERISTICS OF HEPATIC ARTERIAL AND PORTAL FLOWS DURING CEREBRAL ISCHEMIC PRESSOR RESPONSE. TAKEUCHI, T., TERADA, N. and KOYAMA, S. Dept. of Physiol., Yamanashi Medical College. Tamaho, Yamanashi 409-38

Effects of sympathetic nerve activity, hemorrhaging and volume loading on hepatic hemodynamics during cerebral ischemic pressor response were studied in rabbits. The pressor response was produced by stepwise elevation of side pressure on the common carotid artery surgically restricted as a sole route of blood supply to the brain. The hepatic arterial flow recorded with an electromagnetic flowmeter increased initially as systemic arterial pressure rose, which was followed by a decrease at mean systemic arterial pressure above 140 mmHg under normovolemic state. The portal flow only decreased at mean systemic arterial pressure above 100-110 mmHg. Hemorrhaging decreased the portal flow but either increased or slightly decreased the hepatic arterial flow. The hemodynamic patterns of both the blood flows during pressor response after hemorrhaging resembled those under normovolemic state. Volume loading increased markedly the portal flow and generally reduced the hepatic arterial flow, and depressed the increase in the latter that appeared with moderate pressor response. Above-mentioned hemodynamic changes in both the blood flows may reflect the effects of systemic arterial pressure rise and changes in the portal flow on the hepatic arterial flow and sympathetic vasoconstriction.

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EFFECTS OF ELECTRICAL STIMULATION OF THE LIMBIC SYSTEM ON CARDIAC ARRHYTHMIAS INDUCED BY HYPOTHALAMIC STIMULATION. TASHIRO, N., NAKAO, H. and TANAKA, T.*+ Dept. Neuropsychiat., Fac. Med., Kyushu Univ. 60, Fukuoka 812, and + Sch. Nur. Technol., UOEH, Kitakyushu 807.

As little is known of the relationship between emotional behavior and cardiac function, we studied the electrophysiological events during and just after electrical stimulation of the anterior hypothalamus (the AH stimulation), because cardiac arrhythmias induced by the AH stimulation occur simultaneously in alert cats which show restlessness or threat-response (Tashiro, et al., 1981). It is well-known that these emotional behaviors are under the control of the limbic system (especially the amygdala and the septum). Therefore, we attempted to determine whether or not the simultaneous stimulation of the limbic system and the hypothalamus (the AH-LS stimulation) would affect the number of hypothalamus-induced arrhythmias in anesthetized cats. The AH stimulation or the AH-LS stimulation was alternetely applied every ten min. The increase or the decrease in the number of arrhythmias resulting from the AH-LS stimulation was compared with the numbers induced by the AH stimulation, before and after the AH-LS stimulation. The anterior amygdala and the anterior part of the septum had facilitatory effects while the caudal areas of both the amygdala and the septum had inhibitory effects.

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DOES CARDIOVASCULAR REFLEX ARC OF WKY DRIVE RELEASE OF VASOPRESSIN LESS THAN THAT OF SHR DOES? NAKAI,M., YAMANE,Y., and OGINO,K. Natl. Cardiovasc. Ctr, Suita, Osaka 565, and Dept. of Med., Kansai Univ. Med. Sch., Moriguchishi, Osaka 570

Little has been known whether or not is there a difference between SHR and WKY in releasing vasopressin (VP). To answer this issue, experiments were conducted as followings on acutely cordotomized (C-2) rats of 8 week-old under N_2O-anesthesia and artificial ventilation. Rate of iv phenylephrine infusion was adjusted so as to keep arterial pressure at animal's normal level. First, plasma VP concentration was determined under electrical stimulation of the nucleus tractus salitarius (NTS) in rats with additionally dissected vago-sympathetic trunks (VST). We found that in order to reach a level of 137 $\pm$ 37 SE pg/ml, an intensity of stimulus of 128 $\pm$ 60 uA was required in 7 WKYs, whereas to reach a level of 157 $\pm$ 57 pg/ml, an intensity of 39 $\pm$ 5 uA was required in 10 SHRs. Second, we found that acute bilateral ligation of the common carotid arteries, VSTs, or both resulted in an increase of plasma VP concentration by 63 $\pm$ 12 (n=7), 39 $\pm$ 9 (9), or 359 $\pm$ 112 pg/ml (6) in WKY, respectively, whereas 42 $\pm$ 22 (6), 29 $\pm$ 11 (10), or 252 $\pm$ 42 (6) in SHR, respectively. We conclude: (a) the central neural integration originating from NTS regulates release of VP 1/3 less in WKY than in SHR; and (b) an inhibitory tone of carotid sinus baro-, ventricular volume or atrial stretch receptor does not contribute to such a difference.

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INTERACTION BETWEEEN ENKEPHALINS AND CENTRAL ALPHA ADRENOCEPTORS ON CARDIOVASCULAR HEMODYNAMICS IN ANESTHETIZED DOG. KOYAMA,S., TAKEUCHI,T. and TERADA,N. Department of Physiology, Devision 2, Yamanashi Medical College, Nakakoma, Yamanashi 409-38

This study was designed to determine the cardiovascular effects of enkephalins interacted with central alpha adrenoceptors in pentobarbital anesthetized dogs. Mean blood pressure, heart rate and renal blood flow were measured simultaneously. Leucine- and methionine-enkephalins were administered intravenously at the dose of 30 mcg/Kg. Both enkephalins produced the significant reduction in blood pressure, heart rate and renal blood flow for 1-1.5 min after the injection, which returned to control level within 3 min after the injection. Following the intracisternal pretreatment with yohimbin(0.5mg/Kg), decreases in blood pressure and renal blood flow occured by both enkephalins were significantly inhibited, but not in heart rate. And also, these changes in all parameters were completely blocked by intracisternal pretreatment with naloxone(0.2mg/Kg). However, when prazosin(0.1mg/Kg) was pretreated intracisternally, alterations in cardiovascular parameters due to both enkephalins did not significantly differ from the control group. Therefore, these data indicate at least that intravenously administered enkephalins cause the change in blood pressure interacting between opiate receptors and presynaptic alpha adrenoceptors in the central nervous system, parcipitating in central blood pressure regulatory network.

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CARDIAC SYMPATHETIC NERVE ACTIVITY AND HEART RATE VARIATIONS DURING SLEEP AND WAKEFULNESS. NINOMIYA,I., NISHIURA,N., KUZIME,K. AND SADA,K. Dept. of Cardiac Physiol., Research Institute National Cardiovascular Center, Fujishirodai 5-125, Suita, Osaka 565

Using a multichannel telemetory system, cardiac sympathetic nerve activity (CSNA), ECG, heart rate (HR),EMG and EEG were recorded simultaneously continuously over a period of 24 hours in 15 cats. At the onset of mental and physical activities, the grouped CSNA synchronous with cardiac and respiratory cycles reduced and tended to disappear, and futhermore a clustered fluctuation consists of 2 - 16 CSNA waves with intervals of 5 - 15 seconds was frequently observed. During sleep mean CSNA and HR decreased while their respiratory changes increased. Mean CSNA decreased frequently almost to the noise level with or without the rapid eye movements (REM). Duration of CSNA inhibition ranged from 1 to 14 minutes (mean 4.6 min.). During this CSNA inhibition the amplitude of EEG and HR decreased. Howevr, in some cases, HR increased, although mean CSNA inhibited completely. This showed that vagal nerve activity decrased during deep sleep . Immediately after inhibition, in many cases, CSNA and HR increased significantly with overshoot responses.

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DOES SINO-AORTIC DENERVATION RESULT IN A SUSTAINED HYPERTENSION IN RABBITS? SAITO, M*, NUMAO, Y. and KUMADA, M. Inst. of Basic Medical Sciences, The University of Tsukuba, Ibaraki-ken 305

The effect of sino-aortic denervation on the long-term control of arterial pressure (AP) was investigated in eight conscious unrestrained rabbits. An indwelling polyvinyl catheter was placed in the thoracic aorta and was connected to a transducer through an extension tubing and a hydraulic swivel. Mean arterial pressure (MAP) was monitored uninterruptedly up to ten weeks after denervation. The MAP signal was fed into a minicomputer for determination of the mean and standard deviation (SD) of MAP for each day. In seven rabbits, increases in the mean and SD of MAP appeared within 24 hours following denervation. In one rabbit, MAP was abnormally high during the control period and denervation augmented the SD only. In six rabbits out of seven, MAP returned to the control level within five to 50 days after denervation, although the SD remained augmented. In one rabbit, MAP remained elevated throughout the 15-day observation period after denervation. These results support the view that the neurogenic hypertension subsequent to sino-aortic denervation is temporary and that continuous monitoring of AP and computerized averaging procedures are indispensable to investigate the role of the arterial baroreceptors in the long-term control of AP.

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CARDIOVASCULAR RESPONSE TO SHAKING IN THE RAT. TERANISHI, Y., SAKATA, S., and IRIUCHIJIMA, J. Dept. of Physiol., Sch. of Med., Univ. of Hiroshima, Hiroshima 734

Conscious rats chronically implanted with a flow probe and an arterial cannula was shaken on an almost sinusoidal, to and fro shaker at about 3.8 Hz with a peak to peak amplitude of 32 mm. The induced cardiovascular change was referred to as 'shaking response', which was compared with the transposition response induced by transposing the rat to a new habitat. Shaking response was a pressor response in contrast to the almost isopressor transposition response. Cardiac output was increased in both responses, but total peripheral resistance remained almost unchanged in shaking response and decreased in transposition response. Peripheral flow measurements at the superior mesenteric artery and the terminal aorta revealed that the increase in superior mesenteric resistance was greater and the decrease in hindquarter resistance was less in shaking response than in transposition response. These differences were ascribable to that vasoconstrictor fiber excitation was greater and adrenomedullary release was less in shaking response than in transposition response.

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ROLE OF ADRENAL MEDULLA IN MAINTAINING RESTING LEVEL OF ARTERIAL PRESSURE IN CONSCIOUS RATS. IRIUCHIJIMA, J., TERANISHI, Y., and SAKATA, S. Dept. of Physiol., Sch. of Med., Univ. of Hiroshima, Hiroshima 734

In conscious resting rats tonic adrenomedullary secretion keeps muscle blood vessels dilated and heart rate somewhat accelerated. In order to observe the effect of the tonic secretion on arterial pressure, phentolamine(10mg/kg) was injected into rats in which arterial pressure was being recorded continuously. Arterial pressure was lowered from an average of about 110 mmHg to about 80. In adrenalectomized rats the lowering of pressure on phentolamine was only from 110 to 100. At first glance this result seems to indicate that not the tonic vasoconstrictor fiber activity but the tonic adrenomedullary secretion plays the major role in maintaining the resting level of arterial pressure. However, on ganglion blockade or after spinal transection, the difference in arterial pressure between intact and adrenalectomized rats was significant but not so marked as after phentolamine. The tonic adrenomedullary secretion plays a substantial, if not the major role in maintaining arterial pressure in conscious rats. After phentolamine in intact rats, the beta effect of adrenaline, reflexly secreted, seems to dilate muscle blood vessels.

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EFFECTS OF LOW OXYGEN INHALATION ON CARDIAC MUSCLE BLOOD FLOW IN SHEEP UNDER AWAKE CONDITIONS. SAKAI,A., UEDA,G., FUKUSHIMA,M.*, YASAKI,K.*, SHIBAMOTO,T.*, YOSHIMURA,K.*, KUBO,K.*, KOBAYASHI,T.* and SAITO,T.** Dept.of Adapt. Physiology, Dept of 1st Medicine*, Shinshu Univ. Sch. Medicine, Asahi, Matsumoto, 390, BMS**,Kanazawa, 920-02

To elucidate the mechanism of right ventricular hypertrophy (RVH) in response to high altitudes, hypoxia effects of 10 % oxygen inhalation on cardiac muscle blood flow were investigated for sheep (n=5). Specially-made electrodes of an electrolytic regional blood flowmeter (BMS,RBF-1) were impaled to the muscles. It was thus shown that the left ventricular flow ($L\dot{V}Q$) was 93.4 ± 41.1 ml/100g/min for the control and 89.9 ± 38.5 for the hypoxia. Also, the right one ($R\dot{V}Q$) was 69.5 ± 16.0 for the control and 79.8 ± 19.0 for the hypoxia. The latter indicated 15 % increase of flow, whereas the former remained nearly unchanged. From these, it follows that the ratio, $R\dot{V}Q/L\dot{V}Q$, increased from 0.74 at the control to 0.89 at the hypoxia. Corresponding to this, pulmonary artery pressure rise after 10 % O_2 inhalation was noticed whereas systemic artery pressure remained unchanged. These must be the bases for the high altitude RVH.

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TIME-VARIANT PERMEABILITY THEORY ON HIGH-ALTITUDE PULMONARY EDEMA MODEL. UEDA,G,. SAKAI,A., TAKEOKA,M., KOBAYASHI,T.*, KUBO,K.*, FUKUSHIMA,M.*, YOSHIMURA,K.* and SHIBAMOTO,T.* Dept.of Adapt. Physiology, Dept.of 1st Medicine*, Shinshu Univ. Sch. Medicine, Asahi, Matsumoto, 390

Awake standing sheep were exposed to a simulated altitude of 6,600 m for 3 hrs, as high-altitude pulmonary edema condition. The lung lymph flow was designated as JL. The pulmonary transvascular pressure difference ΔP and the osmotic one $\Delta\Pi$ formed forces X. The pulmonary blood and lymph vessel permeability coefficient Lp and solute diffusion coefficient ω formed parameters Y. Thus JL was obtained by X x Y, which indicated that both parameters were time-variant and not constant. The reflection coefficient σ was in limited time periods constant, but in other cases considered as time-variant. From these, an effective permeability $Leff^2$ was estimated by $Lp^2(1-\sigma)$, though this was previously caluculated as $Lp \times \omega / Cs$, where Cs is a protein concentration in the interstitium. The former values for example ranged from 0.15 to 0.10 for controls, and from 0.10 to 0.32 for simulated altitude periods. Thus the increase of flow must be due partly to pressures and partly to permeability factors. The result showed that the smaller pores were opened under low-pressure conditions.

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QUANTITATIVE ANALYSIS OF ATP RELEASE REACTION OF BLOOD PLATELETS CONTINUOUSLY DETERMINED WITH FIREFLY LUCIFERASE LUMINESCENCE. HIGASHI, T., HASHIMOTO, M. and ISOMOTO, A. Dept. of Physicochem. Physiol., Med. Sch., Osaka Univ., Osaka. KAKISHITA, E*, UOMOTO, M* and NAGAI, K* Dept. of Int. Med., Hyogo Col. of Med., Hyogo.

Gel-filtered blood platelets suspended in ATP assay mixture are pipetted into a glass cuvette of Lumi-aggregometer(CHRONO-LOG, AHS/Japan). The ATP release reaction is started by adding collagen. The released ATP reacts with firefly luciferase. The chemi-luminescence is continuously detected and recorded with Lumi-aggregometer. The time-course analysis is tried on this data under the condition as follows. The released ATP is consumed by luciferase enzyme reaction, and its concentration is sufficiently small as compared with Michaelis constant, K_m. And luciferase enzyme reaction is inhibited by oxyluciferin, one of the products. When $f(t)$ is the total amount of released ATP at time t, $[S_t] = f(t) - [I_t]$, and $d[I_t]/dt = v_t = V_{max}[S_t]/K_m(1+[I_t]/K_i)$. Where V_{max}, K_m and K_i are constants, v_t is the reaction rate, and $[S_t]$ and $[I_t]$ are the ATP and oxyluciferin concentration at time t respectively. So, $f(t)$ is expressed as follows, $f(t) = v_t \cdot K_m(1+[I_t]/K_i)/V_{max} + [I_t]$. If the recorded data, v_t and $[I_t]$ are substituted into this equation, $f(t)$ is able to be determined quantitatively and continuously.

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EFFECTS OF CATECHOLAMINE ON PLATELET AGGREGATION IN RAT AND RABBIT. TANIMOTO, Y. and HATTORI, Y. Dept. of Hematochemistry, Cent. Inst. for Experimental Animals, Miyamae-ku, Kawasaki 213

ADP-induced platelet aggregation was increased in the presence of epinephrine (2-20μM), markedly in rabbits and slightly in rats. Collagen-induced platelet aggregation was unaffected by epinephrine and norepinephrine in rats and rabbits. Collagen-induced platelet aggregation decreased in the presence of phentolamine and propranolol in male and female rats especially with the former, which showed significant sex difference. Isoproterenol inhibited collagen-induced platelet aggregation, slightly in female rats, severely in rabbits. The α and β antagonist labetalol inhibited the aggregation of male rat and rabbit platelets by collagen, with no effect in female rats and with significant difference between the male and female rats.

In conclusion, while ADP-induced platelet aggregation is associated with α-adrenergic effects in the rabbit, the inhibition of collagen-induced platelet aggregation in both animals is chiefly related to the irritation of β-adrenergic receptors.

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ON THE LOW CLOTTABILITY OF FETAL BLOOD. HASEGAWA, N., NAKASHIMA, A. and SASAKI, S. Department of Physiology I, Fujita-Gakuen University School of Medicine, Toyoake, Aichi, 470-11.

The study was performed to elucidate the cause of low clottability of fetal blood compared to the adult blood. Electrophoretic patterns of the two plasmas are quite different from each other. This is mainly due to the higher concentration of a non-clottable protein having mobility similar to that of HMW-fibrinogen in fetal blood than in adult blood. This protein was identified to be alpha-2-macroglobulin by immunological method. Purified alpha-2-macroglobulin separated from adult plasma by affinity chromatography gave the same mobility as HMW-fibrinogen. When this globulin was added to adult fibrinogen at the concentration similar to fetal blood, clotting time was prolonged as expected. The prolongation of clotting time of plasma after addition of the macroglobulin was effected by the concentration of calcium ion. It is concluded that alpha-2-macroglobulin seems to be a part of the cause of the low clottability of fetal blood compared to adult blood.

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THE INHIBITORY EFFECT OF GARLIC OIL ON PLATELET AGGREGATION (III)—ORAL ADMINISTRATION IN RABBITS—

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In the present investigation, we attempted to clarify the in-vivo effect of garlic oil on the platelet aggregation in rabbits. Garlic oil of 0.07 ml/kg and 0.14 ml/kg doses were given orally to two groups of rabbits respectively. Each group was comprised of 7 to 12 animals. The platelet aggregation was tested on the PRP prepared prior to 3, 6, 24 and 48 hours following the administration of the garlic oil. The aggregation inducers used were ADP (20 μM), arachidonic acid (AA)(0.25 mM) and collagen (6 μg).
1) In the low dose experiments (0.07 ml/kg), no effect was observed on the aggregatory process of the platelets, while a tendency of facilitation on the dissociation of ADP-induced aggregates was observed at 3 hours and 6 hours after the oil ingestion.
2) In the higher dose experiments (0.14 ml/kg), the evident inhibition was seen at 3 hours and 6 hours after the oil ingestion in the platelet aggregation challenged with any inducers employed: the percent inhibition was 31%~46%. Such inhibition was observed to be lost after 24 hours in ADP induced aggregation, while it lasted for 24 hours in collagen-induced aggregation, and 48 hours in AA-induced aggregation.

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H-NMR-SPECTRUM OF WATER IN BLOOD COAGULATION AND FIBRINOLYSIS.

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Behavior of water in fibrinogen solution (Fbg), fibrin-gel (Gel) and lysed gel (Lys) was taken with 80MHz high resolution FT-NMR spectrometer (Bruker, WP 80 SY-WG). Fbg preparations in 3 kinds of final concentration were made to be 1, 5, and 10 mg of bovine fibrinogen (Miles)/ml of borate saline buffer (pH=7.75) containing 0.5 μl of pooled human serum (from clinic of our hospital) and 10 U of activator SK (Varidase, Lederle). To each of those samples, 1 drop of borate buffer solution of Mochida's Thrombin was added for the Gel preparation which was made into the Lys preparations by standing at 24°C of R.T. for about 50 minutes. Each preparation containing 10 % D_2O was sampled into 5mm-NMR-tube and showed the peak at 1687Hz in H-NMR-spectrum which decreased in the intensity (peak height, h) so much proportionally as the fibrinogen concentration increased, but not so much in the integral value (S). Then, the values S/h especially in Gel samples were increased proportionally to concentration of fibrinogen, but little in the Fbg and Lys samples. In other words, it means that water signal of the Gel in the width is dependent upon the concentration of fibrinogen.

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STUDIES ON TWO ISOZYMES OF GLU-PLASMINOGEN(I AND II): STRUCTURE AND ACTIVITY.

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Glu-plasminogen (Glu-plg) was eluted through lysine-Sepharose by using a gradient of 6 aminohexanoic acid (6AHA), and two peaks corresponding to Glu-plg I and II were obtained. Glu-plg I has a molecular weight of 93,000 and Glu-plg II has a molecular weight of 89,000. When these plgs were activated by urokinase (UK) or streptokinase (SK) in the presence of S-2251 (H-D-Val-Leu-Lys pNA), the hydrolysis of S-2251 by Glu-plg I activated by UK or SK was larger than that by Glu-plg II activated by UK or SK. The results of SDS-PAGE indicate that the conversion of Glu-plg I to plasmin by UK was faster than that of Glu-plg II. It may be concluded that Glu-plg I is activated better to plasmin by activators than Glu-plg II. Structural basis for such differences between Glu-plg I and II was studied by using fluorescence spectrophotometer and circular dichroidism. Glu-plg I changed the conformation in larger extent than Glu-plg II when tranexamic acid or 6AHA bound with its lysine-binding sites. These observations indicate that Glu-plg I is more flexible than Glu-plg II.

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EFFECTS OF LYSINE OR ITS ANALOGUES ON SUBCOMPONENT C1q OF THE FIRST COMPONENT OF HUMAN COMPLEMENT. TAKADA, A., SUGAWARA, Y. and TAKADA, Y. Department of Physiology, Hamamatsu University, School of Medicine, Hamamatsu-shi, Shizuoka-ken, Japan 431-31

Lysine or its analogues were tested for their capacities to inhibit the activation of complement by heat aggregated immunoglobulin (aggIgG), the consumption of complement by aggIgG, the inhibition of hemolysis of erythrocytes bound with hemolysin (EA), and C1 hemolysis. The aminoacids used were lysine, arginine, histidine, glutamic acid, glycine, 6 aminohexanoic acid (6AHA), norleucine and tranexamic acid. Lysine was most effective in the inhibition of complement activation which was followed by arginine. Glutamic acid was effective although less than lysine in the inhibition of C1 hemolysis. 6AHA or tranexamic acid, which did not inhibit C1 activation at low ionic strength at 30 mM, effectively inhibited C1 hemolysis, suggesting that these ω-aminoacids prevented the activation of C1 by antibody molecules more effectively than the conformational change of C1q at low ionic strength. Norleucine (α-aminocaproic acid) was hardly effective, thus ε-aminoradical of lysine was more important than its α-aminoradical. Tuftsin which was proposed to comprise C1q binding site inhibited C1 hemolysis twice as effectively as lysine.

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VARIATIONS OF SERUM α-CYSTEINE PROTEINASE INHIBITOR LEVEL. MINAKATA, K. Dept. of Legal Medicine, Hamamatsu University, School of Medicine, 3600 Handa, Hamamatsu 431-31

Variations in α-cysteine proteinase inhibitor(α-CPI) level were investigated using our method reported previously[1]. α-CPI level in 111 pregnant women increased as the pregnancy advanced. The correlation coefficient between the level and the duration, r, was 0.578 with the level of significance $p<0.001$. The mean value of 50 pregnant women in the third trimester of gestation was 0.49 unit/ml, which was 47 % higher than the control value. On the contrary, α-CPI levels of the vitamin E deficient rats was 5 to 7 fold of the control value. The correlation coefficient between α-CPI level and serum vitamin E level was -0.962 with the level of significance $p<0.001$. The strong correlation between vitamin E level and α-CPI level may suggest that α-CPI level can be used as a more sensitive index than serum GOT level used previously to represent the vitamin E deficiency.

[1]. Minakata, K., Asano, M., Sato, T. and Harada, N. Hoppe-Seyler's Z. Physiol. Chem. 363, 493 (1982)

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THE INTERACTION OF HUMAN PLASMINOGEN AND PLASMIN WITH SK OR OZONE TREATED SK, AND THE FUNCTIONS OF SK FRAGMENTS. YAMAMOTO,H., NAKASHIMA,A., SAKAI,J., SAEDA,Y. AND SUGIE,I. Dept. of Physiology, Aichi Medical University, Nagakute-cho, Aichi-gun, Aichi-ken

The interaction of human PLG and PL with SK was studied using normal and SDS-polyacrylamide gel electrophoresis and immunochemicalelectrophoresis methods, after incubation of SK and PLG, PL at 37°c for 5 minutes. The reaction system which molar ratio of SK to PLG (SK:PLG=1:1, 28:1 SK:PL=1:1,20:1) was selected. In any reaction system,equimolar complex of SK and PLG, PL was formed and PLG, PL, SK undergoes progressive degradation. These complex activated PLG to PL and was active tword both kinds of synthetic substrates S-2251, S-2444. SK degradated product did not activate PLG nor inhibit PL. These antigenesity of SK was decreased. SK is a single chain protein and contains only one tryptophan residue, no cysteine residues. Tryptophan residue in SK molecule was modified by ozone treatment. This modified SK could activate PLG to PL. So we conclude the tryptophan residue of SK does not constitute part of the binding site for PLG.

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RELATIONSHIP BETWEEN OXYGEN UPTAKE LEVELS AND CHANGES OF URINARY COMPOSITIONS DURING EXERCISE.

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The relation between the oxygen uptake levels and the changes of urinary compositions during exercise on a bicycle ergometer was investigated in eight male athletes.

Urinary Na^+, Cl^-, and K^+ increased after exercise which was less than 60 % $\dot{V}O_2$ max, but evidently decreased after exercise which was more than 70 % $\dot{V}O_2$ max.

The post/pre- ratios of Na^+, Cl^- and K^+ (30 minutes after exercise urine/pre-urine) showed inverse correlation with % $\dot{V}O_2$ max ($p<0.01$).

The cumulative excretion rates of Na, Cl, K and urinary volume, up to 120 minutes after high intensity exercise showed decrease than that of pre-exercise. The excretion rate of creatinine was decreased temporarily after high intensity exercise.

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Regulations of body fluid and circulation during and after thermal dehydration in men.

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Body fluid and circuratory regulations were studied during and after thermal dehydration in men. Subjects were nine healthy males (21~33 years). They sat in a chamber (65~70°C) for 30 min, and recovered in a room (20~22°C) for two hours. Significant decrease in enddiastolic pressure and dilatation of peripheral vessels in finger (plethysmography) were observed following the exposure to hot environment. Increase in oxygen consumption was observed very slightly. Mean body weight loss was 0.38 kg. Urinary volume for 30 min. after thermal load decreased to 42 % of the initial level. It did not return to the pre-stress level after two-hour recovery even though it increased gradually. Although serum protein, Na^+, and K^+ concentrations were increased following thermal stress, serum osmotic pressure did not change significantly. The concentrations in plasma renin, aldosterone, angiotensin II, and ADH were elevated but the elevations in plasma adrenaline and noradrenaline were not significant. Neither the changes in plasma volume nor serum osmotic pressure have direct relationship with ADH secretion. But exponential relationship was observed between urinary volume and plasma ADH level. ADH secretion was remarkable when the urinary volume was less than about 0.3 ml/min.

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EFFECTS OF METHYLPREDNISOLONE ON WATER TRANSPORT IN PROXIMAL STRAIGHT TUBULES IN VITRO

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We have investigated the change of intrinsic water transport capacity in proximal straight tubule (PST) in vitro which is stimulated by loss of unilateral kidney excretory function induced by unilateral nephrectomy, unilateral ureteral obstruction or unilateral ureteroperitoneostomy, concomitant with increase of glomerular filtration rate (GFR). To investigate further the relationship between GFR in vivo and water reabsorption rate (Jv) in vitro, Methylprednisolone (MP) which is known to increase GFR was injected to New Zealand white rabbits, 15mg/kg for 5 days. After the confirmation of increase of GFR by clearance method, in vitro isolated tubular microperfusion was performed to evaluate the change of intrinsic water transport capacity in PST. MP administration increase GFR by 73%. MP induced cellular hypertrophy and enhanced water reabsorption in PST by 28%. Correlation between GFR in vivo and delta increase of Jv in PST suggested that the change of intrinsic water transport capacity stimulated by the increase of GFR which are induced whether by loss of unilateral kidney excretory function or by MP administration, might play an important role to maintain the "glomerulo-tubular balance".

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EFFECT OF COMPOUND 48/80 ON WATER INTAKE IN THE RAT. IZUMI, H and AOKI, T. Department of Physiology, Tohoku University School of Dentistry, Seiryo-Machi, Sendai 980

The subcuteneous administration of compound 48/80 (C-48/80, a histamine liberator) in the rat produced a long-lasting increase of water intake, accompanied with remarkable increase of plasma renin activity (PRA) and hematcrit. The increase of PRA and that of hematocrit were both inhibited by tripelennamine (H1-blocker), but not by cimetidine (H2-blocker) or propranolol (β-adrenergic blocker). The onset of hematocrit increase was found to precede that of PRA increase. Nephrectomy nullified the water-intake-stimulatory effect of C-48/80 without affecting the hematocrit-increasing effect. Histamine itself also induce increase of water intake with rapid onset and short-lasting, without increasing PRA and hematocrit. This indicate that the mechanism of water intake increase by C-48/80 differ from that by histamine. Our results suggest that C-48/80 releases histamine from mast cells at the injection site and causes plasma extravasation. The resulting hypovolemia stimulates juxtraglomerular cells of kidney to release renin, which in turn triggers drinking.

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BEHAVIOR OF WATER SIGNAL IN URINE COMPONENTS BY H-NMR. NAKAYA, S., TANAKA, Y., HONJO, R. and YAGI, S. Dept of Physiol., Sch. of Med., Iwate Med. Univ., Morioka.

High Resolution H-NMR (BRUKER WP 80 SY-WG, 18.8 kGauss) was used to analyse the water signals (Free Induction Decay FID in the time domain; Fourier Transform FT-spectra in the frequency domain; Inversion Recovery T1 value) which were produced at resonance frequency of 80.13 MHz by different components of human urine (NaCl, KCl and urea solutions) each in 5 mm NMR-tube of 1 ml aliquot containing 0.1 ml of D_2O at 24°C.

Each FT-spectrum band of water with a single peak of 1687 Hz with a variety of width and height was so much reduced in the integral value but not so much in the intensity (peak height), as each component was concentrated into several steps to about 50 %. The reductions dependent upon the concentration were larger in KCl than those in NaCl and urea. Those among normal samples of human urine with about 6 of pH and 1.015 to 1.026 of specific gravity were plotted into the ranges of 2 to 3 % NaCl, respectively. The varieties in the FT-spectrum band of water were related propotionally rather to those in oxygen tension than to those in pH. T1 values among samples examined were not significantly different from those of pure water.

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RELATION BETWEEN THE CROSSLINKING OF MEMBRANE PROTEINS AND THE RHEOLOGICAL FUNCTIONS OF HUMAN ERYTHROCYTES. MAEDA, N., IMAIZUMI, K., KON, K. and SHIGA, T. Dept. of Physiol., Sch. of Med., Ehime Univ., Shigenobu, Onsen-gun, Ehime 791-02

The cytoskeletal protein, spectrin, was specifically crosslinked by a SH-oxidizing agent, diamide, and the effect of crosslinking on the rheological functions of human erythrocytes was examined. (1) The suspension viscosity of erythrocytes increased proportionally to the degree of crosslinking (quantified by polyacrylamide gel electrophoresis in 1 % sodium dodecyl sulfate). (2) The decreased deformability of erythrocytes was observed by a high shear rheoscope and was detectable at 5 % crosslinking. (3) The decreased rate of rouleaux formation (measured by a TV-image analyzer combined with a low shear rheoscope) was sensitively detected at more than 5 % crosslinking. (4) The membrane fluidity in the middle portion of lipid bilayer (measured by the spin label method) was significantly decreased by the crosslinking (5) The increased suspension viscosity and the decreased deformability were reversed by the reduction of disulfide bridges with dithiothreitol, though not complete.

The organization of the cytoskeletal structure of membrane is important for the rheological functions of erythrocytes, and the present results can estimate the pathophysiological meaning for crosslinking of erythrocyte membrane proteins.

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KINETICS OF ERYTHROCYTE ROULEAUX FORMATION USING TV IMAGE ANALYZER. III. KINETICS.
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In order to elucidate the formation mechanism of erythrocyte rouleaux in shear flow, the development of new apparatus for studying basic kinetic processes is needed. A rheoscope combined with TV image analyzer and computer (this journal,44,22,187,496,'82) is suitable for observing the time course and analyzing the basic processes.

SPF rat blood (containing little γ-globulin) is adequate sample for kinetic study, because only one dimensional rouleaux is formed without developing three dimensional aggregates. The observed phenamena could be expressed by the following parameters. (1) Sedimentation rate constant: erythrocytes sedimented in the apparatus following two compartments approximation. (2) Association rate constant of erythrocyte linear polymerization: according to the theory of Oosawa and Kasai (J.Mol.Biol.,4,10,'62) neglecting the dissociation constant. (3) Changes in projected areas: due to random motion of monomeric erythrocytes and due to rotation of polymeric erythrocytes.

Combining these rate processes, the experimental results could be successfully simulated by computer. The acceleration of rouleaux formation due to increase of hematocrit and of plasma proteins (fibrinogen, γ-globulin, etc) is interpreted. The Poissonian distribution of erythrocytes in rouleaux is explained by theory.

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EFFECTS OF IMMUNOGLOBULINS AND IgG-RFAGMENTS ON THE AGGREGATE FORMATION OF HUMAN ERYTHROCYTES.
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Effects of immunoglobulins and IgG-fragments on the aggregate formation of human erythrocytes were investigated, using a rheoscope combined with a TV image analyzer and computer. The trend of the increased velocity of the reversible, non-immune aggregation by immunoglobulins was IgG ≃ γ-globulin < IgA < IgM, corresponding to the increase in their molecular weights. This may be explained by (1) an increase of the number of sites attaching with erythrocyte surface per immunoglobulin molecule and (2) the reduction of the electrostatic repulsion of the erythrocyte surface due to the increased intercellular distance (at the binding position). A comparison among IgG-fragments on the aggregate formation revealed that only $F(ab')_2$ increased the velocity, while Fab and Fc showed no acceleration. Moreover, $F(ab')_2$ was more effective than IgG (about twice). However, IgG-induced aggregation was inhibited by the addition of Fab or Fc. The degree of the inhibition by Fab was 1.6 times stronger than that of Fc. Therefore, on the aggregation of erythrocytes, two Fab portions of immunoglobulins play an important role for the bridging between adjacent cells, and also Fc portion contributes in lesser extent.

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EFFECTS OF A CALMODULIN ANTAGONIST ON THE RHEOLOGICAL PROPERTIES OF UNSEALED ERYTHROCYTE GHOSTS.
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Role of calmodulin (CaM) in regulation of rheological properties of erythrocyte membranes was examined by using a CaM antagonist, W-7. When observed by phase contrast microscopy, unsealed freeze-thawed erythrocyte membranes appeared as smooth cup shaped membranes. Application of W-7 to these membrane preparations resulted in a change of the shape appearing more spherical cups. Exposure to W-7 caused a decrease in viscosity of the suspension of unsealed membranes. Based on the viscosity - rate of shear relationship of the suspensions, it is concluded that decreased suspension viscosity due to W-7 does not reflect changes in membrane deformability but does those in membrane shape. Action of W-7 to decrease viscosity of the suspension of unsealed membranes was depressed in the presence of excessive calcium-activated CaM. These results appear to show that CaM is related to the regulation of erythrocyte membrane shape.

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ANALYSIS OF ARTERIOLAR RED BLOOD CELL VELOCITY AND DYNAMIC HEMATOCRIT IN RABBIT MESENTERY.

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Red blood cell(RBC) volume fraction in microcirculation is less than in macrocirculation. Changes of RBC volume fraction (dynamic hematocrit) is more remarkable in arterioles than capillary networks. We measured the changes of arteriolar RBC velocity and flux to analyse dynamic hematocrit. RBC velocity was measured by a dual-slit photometric technique and flux was determined by counting up the peaks of photo-sensor signal due to the passage of each RBC.

It was found that the RBC velocity was linearly increased with systemic blood pressure elevation under the effect of noradrenaline infusion (10 μg/kg i.v.). The regressive line between the systemic blood pressure and RBC velocity in arterioles suggested the existence of critical closing pressure. The RBC flux was also linearly increased with RBC velocity. The relative changes of the dynamic hematocrit in arterioles could be calculated numerically from the RBC velocity and flux. We found that dynamic hematocrit was decreased when the RBC velocity became greater. This result may suggest that the RBC supply to capillary networks is not necessarily increased in spite of increasing of bulk blood flow.

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THE MECHANISMS OF FETAL HEMOGLOBIN (Hb F) REACTIVATION BY ERYTHROPOIETIC PRECURSORS (BFU-E) IN CULTURE. TERASAWA, T and KASAI, S. Dept. of Physiol. Tohoku Dental Univ. Koriyamashi, Fukushimaken, 963.

The effects of burst-promoting activity (BPA) and erythropoietin (Epo) on the Hb F biosynthesis were investigated. Blood mononuclear cells were cultured and labeled with ^{14}C-amino acid on day 12-13. Hb synthesized in the bursts was separated by isoelectric focusing. The relative biosynthetic rates of Hb F were determined by densitometric tracings of autoradiograms. In culture with pure Epo (70,400 U/mg), the percentage of Hb F biosynthesis by BFU-E remained constant between Epo concentrations of 0.25 and 4.0 U/ml. With crude Epo both sheep plasma Epo (14 U/mg) and human urinary Epo (40 U/mg), there were significant correlations between Hb F biosynthesis and Epo concentrations. Thus, the dose response to crude Epo may have been due to non-Epo factors. Next, we examined the effects of marrow BPA and BPA derived from human T-cell line on Hb F biosynthesis in culture. The percent enhancement of the relative rates of Hb F biosynthesis by both BPA was 52 ± 21%. We, therefore, conclude that BPA are important in the modulation of Hb F biosynthesis in culture.

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SIMULATION AND PREDICTION OF PERIPHERAL BLOOD CELL DYNAMICS IN ACUTE LEUKEMIA BY MATHEMATICAL MODELS. MORI. H., TAKEKAWA, A. and KITAMURA, S.* Dept. of Physiol. Kobe University School of Med. Chuo-Ku, Kobe 650 and Dept. of Instrumentation Engineering, Faculty of Engineering, Kobe University, Nada-Ku, Kobe 657.

The dynamics of the peripheral blood cell counts during treatment in acute leukemic patients was described by mathematical models. A differential equation model was able to fit and predict the dynamic relationship between the input variables, administered anti-leukemic drugs and the output variables, the number of leukemic cells or normal white blood cells. The numerical values estimated from eleven patients indicated that the leukemic cells increased more rapidly and reacted more sensitive to the drugs than the normal white blood cells.

The equation is $$\frac{1}{N(t)}\frac{dN(t)}{dt} = a - \gamma D_1 - \frac{\alpha}{\beta}\left(1-\exp^{-\alpha D_2}\right)$$

where N(t) is the number of cells, D_1 and D_2 are the concentration of drugs representing two kind of mode of action and a, γ, α and β are the parameters to be estimated by a least square method.

On the other hand, the dynamics of platelets whose response to the drugs was not confirmed quantitatively was simulated by the Auto-Regression model.

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Effects of acetazolamide and SITS on the appearance of Na^+ and HCO_3^- from red blood cells into the plasma during the passage of CO_2-rich gas.

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We have previously reported that, very probably, there occurs an almost reversible "transport" of Na^+ and HCO_3^- from red blood cells into the plasma side-by-side with the well-known chloride shift, when dog's whole blood was exposed to the CO_2-rich gas mixture (60% N_2,25% O_2,15% CO_2).

In the present study, we tried to investigate the mechanism involved in this ion "transport" in question. For this purpose, dog's whole blood was pretreated with acetazolamide(Az,40 mg/kg,i.v.) or SITS($2x10^{-4}$ Mol/L,in situ) before exposure to the gas mixture discribed above.

(1) Calculated approximate net efflux of Na^+ (FNa, out) and that of HCO_3^-($FHCO_3^-$, out),and also,calculated approximate net influx of Cl^-(FCl,in) that occurs during the first 5 minutes after the start of gas passage, were all decreased after pretreatment with Az or SITS.(2) Sum of the net FNa, out and absolute value of net FCl, in closely agreed with net $FHCO_3^-$, out, suggesting a parallel operation of Na^+-HCO_3^- channel and chloride shift.

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THEORETICAL AND EXPERIMENTAL BACKGROUND FOR EXISTENCE OF BASE EXCESS CURVE.

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Base excess curve on the Siggaard-Andersen's nomogram is a trace of crossing points of buffer curves of a blood sample with the same buffer base value (BE) but with different hemoglobin concentrations (Hb). We have shown in the previous report that a hyperbolic function defining the BE curve on the pH-HCO_3^- coordinates can be derived from the two-compartmental model of blood buffer system. (J. Physiol. Japan 44:492, 1982, Am J. Physiol. 1983 in press) Questions that we posed in this study was twofold: What is the necessary and sufficient condition for the BE curve to be uniquely defined independent of hemoglobin concentration? Is it theoretically reasonable to extend blood BE to the whole extracellular fluid space by diluting red blood cells, e.g. Hb 5 g/dl. The first question was answered by the assumption of two compartments of blood buffer system in which the buffer slopes are different in each compartment. Siggaard-Andersen's own one-compartmental analysis yields a family of BE curves depending upon a pair of Hb of the blood samples. The second question was denied on the theoretical basis, although experimentally the error induced by neglecting Donnan's equilibria through the capillary membranes did not seem significant.

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THE CHANGE OF FREE CALCIUM IONS AND OF CO_2 GAS IN THE BLOOD.

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The effect of pH on the change of free calcium ions in whole blood is of clinical interest. The relationship between free calcium ions and pH in normal blood, plasma and albumine was demonstrated by means of a new method which was developed for the determination of the CO_2 dissociation curves and free Ca change of samples. The change of total CO_2 content, pH, and free Ca vs. pCO_2 of samples were monitored continuously on two X-Y_1,Y_2, recorders. The CO_2 equilibrium curves, their components (physically dissolved CO_2 and plasma bicarbonate), and ionized calcium change (which monitored by potentiometrically) of samples were shown experimentally. The relationship with a slope of d pCa / d pH between free calcium and pH change due to CO_2 titration in normal blood (Hct 45%) and plasma were -0.146 and -0.227 respectively. On the other hand, these values due to acid-base titration were -0.35 and -0.579 respectively. And also they were dependent on the albumin concentration.

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PLASMINOGEN ACTIVATOR SYNTHESIS IN CULTURED ENDOTHERIAL CELLS.
KITAGUCHI, H., OGAWA, K., HIRATA, M., MIKI, M. and FUNAHARA, Y. Dept. of Physiology, Kobe Univ. School of Med., Chuoh-ku, Kobe 650

The plasminogen activator activity has been measured in cultured endotherial cells from human umbilical artery and vein, canine aorta, canine vena cara and canine coronary vessels. The human umbilical endotherial cells were maintained in medium 199 containing 10% human serum, whereas all of the canine origin endotherial cells were grown in medium 199 containing 10% fetal calf serum. Plasminogen activator activity was observed in all of the cultured endotherial cell lysates excepting the lysates of umbilical artery and vein.

These results indicated that cultured endotherial cells from human umbilical artery and vein were unsuitable tool for analysis of plasminogen activator synthesis and secretion.

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RELEASE OF PLASMINOGEN ACTIVATOR OCCURING IN DOGS DURING HYPOTHERMIA,
YOSHIHARA, H., YAMAMOTO, T., MARUYAMA, M., NISHIDA, Y., AKAZAWA, K., SUMI, H. AND MIHARA, H. Dept. of Physiology, Miyazaki Medical College, Miyazaki

The changes in coagulation, platelet and fibrinolysis during experimental hypothermia were studied on mongrel dogs. The animals were anesthetised with pentobarbital sodium and were divided into three groups. Control group(group A) was maintained at 37°C in core temperature. Simple hypothermia group(group B) was cooled at 20-22°C and another hypothermia group(group C) was premedicated with chlorpromazine preventing the ventricular fibrillation.

Blood samples were drawn from superior vena cava through the catheter. The results obtained were as follows: (1) Coagulation: Recalcification time, APTT and PT were measured. In group A and C, coagulation times were progressively shortened. However, there were no significant changes in group B. (2) Platelet function: In group A, no significant changes were observed. However, in group B and C, platelet count and aggregability were progressively decreased during the hypothermia. These reductions were recovered by rewarming. (3) Fibrinolytic activity: In group A, no changes of fibrinolytic activity were observed. On the other hand, in group B, fibrinolytic activity increased during the low body temperature and the rewarming period, but it was not observed in group C.

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GASTROINTESTINAL ABSORPTION OF HIGH MOLECULAR WEIGHT UROKINASE AND ITS HEAVY CHAIN DERIVATIVE.
SUMI,H., MARUYAMA,M., YONETA,T., AKAZAWA,K. AND MIHARA,H. Dept. of Physiol. Miyazaki Medical College, Miyazaki-gun, Miyazaki, 889-16

Following intrarectal administration of high molecular weight urokinase (HMW-UK; mol.wt. 53,000, 124,000 IU/mg protein) or its functionally-active heavy chain (H-chain UK; mol.wt. 31,000, 212,000 IU/mg protein) with gastric mucin to mice, prolonged activation of plasma fibrinolysis was observed. The plasma levels of pyro-Glu-Gly-Arg-pNA amidolysis and euglobulin fibrinolytic activities reached a maximum (43.4 $\pm$ 5.3 n moles/ml and 10.8 $\pm$ 9.4 mm^2/ 0.03 ml, respectively) at 1 to 2 hr after the administration of 300 IU of either enzyme per mouse, and thereafter both activites declined slowly. On the other hand, the plasma coagulation activity by neither APTT or Re-Ca^{++}T showed any significant difference between the control and treated groups. Absorption of the UK was confirmed by pooling the fractions which are eluted by 6 M urea buffer following [N^{α}-(ε-aminocaproyl)-DL-homoarginine hexylester]-Sepharose affinity chromatography. These eluted fractions were shown to have fibrinolytic activity, to form a precipitation arc with appropriate antisera by immunodiffusion and could be neutralised by addition of the antisera in the same way as parent UK. There may be some potential therapeutic importance of these observations although less than 3% of the administrated dose was calculated to reach the circulation.

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STUDIES ON PROTEASE IN VENOM OF TRIMERESURUS FLAVOVIRIDIS. KOSUGI, T., ARIGA, Y., TAKAGI, I. and KINJO, K. Dept. of Physiol., Sch. of Med., Univ. of the Ryukyus, Okinawa 903-01

It has been known that protease contained abundantly in venom of Trimeresurus flavoviridis. In this study, a protease with caseinolytic activity was partially purified using the technique of ammonium sulphate fractionation, gel filtration ion, exchange chromatography.

To purify the caseinolytic protease, crude venom was obtained from the snake, Trimeresurus flavoviridis, for use as the starting material.

The molecular weight of this caseinolytic protease was about 10,4000 daltons. The molecular weight and the behavior to some synthetic inhibitors was similar to those of HRI fraction previously purified by Ohsaka. Inhibitory effect of EDTA, however, to this caseinolytic protease and the effect of metal ions were entirely different from HRI.

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INFLUENCE OF OUABAIN AND ETHACRYNIC ACID ADMINISTRATION ON SALIVARY SECRETION. NEMOTO, A., HOSOI, K. and UEHA, T. Dept. of Oral Physiol., Josai Dental University, Sakado, Saitama

We have investigated the effects of ouabain and ethacrynic acid on electrolyte concentrations and salivary flow rates in the rat (SD strain) submandibular gland. The methods of partial perfusion and intraductal injection were used to administer the two drugs to the gland. When the partial perfusion technique was used, the concentration of Na^+ in saliva increased following administration of ouabain and decreased with ethacrynic acid. And, using the method of intraductal injection, the concentration of Na^+ in saliva decreased and K^+ increased following administration of either drug. The salivary secretion rate was severely depressed following administration of ouabain by the partial perfusion method. We propose the existence of two transport systems. One is the ouabain-sensitive pump which would be located both in the basal and luminal portions. And the other is an ethacrynic acid-sensitive pump existing in the luminal areas.

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DEVELOPMENT OF ON-LINE MEASUREMENT SYSTEM FOR OXYGEN UPTAKE. Ikegami, Y., Miyamura, M. and Matsui, H. Research Center of Health, Physical Fitness and Sports, Nagoya Univ. Chikusa-ku, Nagoya 464

A new system for continuos and automatic measurement of oxygen uptake using minicomputer system was developed. Pulmonary ventilation was measured by the piston type spirometer with rotary-encoder to detect the displacement of the piston proportional to the volume changes. Electric pulse generated from the rotary-encoder was integrated with electric counter and transfered to the computer system. O_2 and CO_2 concentrations in the expired gas were determined with zirconium O_2 gas analyzer and infrared CO_2 gas analyzer and analog signals from these analyzers were inputed to the computer system using A-D converter. O_2 uptake, pulmonary ventilation, CO_2 output etc were calculated by the computer system. Accuracy and reliability of the system were examined compairing with the Douglas bag method. The difference in O_2 uptake obtained by the new system and the Douglas bag method was less than 5% to the extent of 150 l/min of ventilation and 3.5 l/min of O_2 uptake. Main factor of the error depended on the difference of O_2 concentration measured by the Douglas bag method and the new system using mixing chamber. From these results, it was suggested that mixing method should be improved to obtain higher accuracy.

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INDIRECT DETERMINATION OF CARDIAC OUTPUT USING O_2 AND CO_2 CURVES DURING REBREATHING. SHIMASAKI, T.*, TAMURA, M. and MOCHIZUKI, M. Dept. of Physiology, Yamagata University, School of Medicine, 990-23 Yamagata.

Expired O_2 and CO_2 curves were recorded during rebreathing. Gas exchange ratio (RQ) was calculated from both the rates of CO_2 increase and O_2 decrease measured at every inspiratory phase of 10 to 11 breathings. A linear relation was ascertained between the RQ and Pco_2 measured at the same time. Theoretically, the arterio-venous O_2 content difference ($avDo_2$) was given by using both the slopes of RQ/Pco_2-relation (K) and the CO_2 dissociation curve (α') as follows : $avDo_2 = \alpha'/K$. Thus, in order to validate the above theoretical evidence, the $avDo_2$ was measured together with K and α' values in dogs. Between the calculated and measured $avDo_2$'s good correlation was found. In addition, a known amount of pure O_2 was injected in the lung-bag system during the rebreathing, and from the recovery time the O_2 uptake was evaluated. Therefore, by dividing the O_2 uptake by the $avDo_2$, the effective pulmonary blood flow was estimated, which was distributed in a narrow range within 12 % of the measn value in SD. Such accuracy as above was obviously higher than the CO_2 rebreathing and the direct Fick methods and moreover the used technique was simple, hence, it seemed fairly advantageous for clinical purposes.

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MEASUREMENTS OF DISSOCIATION CURVE AND DIFFUSION COEFFICIENT OF CARBON DIOXIDE IN HEMOGLOBIN SOLUTIONS. UCHIDA, K., MOCHIZUKI, M. and NIIZEKI, K. Dept. Physiol., Yamagata University School of Medicine, Yamagata 990-23.

pH changes in a thin layer of hemoglobin (Hb) solutions accompanying a sudden change in Pco_2 of the surrounding gas were followed by pH sensitive fluorescence of 4-methylumbelliferone. The pH-time curves were converted into Pco_2 curves according to the observed linear relation between pH and log Pco_2. The pH rise observed in the CO_2 diffusion out of the layer was slower than the pH fall, while the outward diffusion rate was faster than the inward diffusion rate. The CO_2 contents of Hb solutions were measured by using a Natelson's blood gas analyzer and the slope (α') of the dissociation curve was obtained as a function of Pco_2 and Hb concentration : $\alpha' = 9.43 \times 10^{-3} \cdot B \cdot (Pco_2/77.5)^{B-1}$ $Torr^{-1}$, $B = 0.0406 + 0.594[Hb]$, where [Hb] represents a fractional hematocrit value before hemolysis. In order to simulate the Pco_2-time curves the diffusion equations which included the non-linear α' were numerically solved. The diffusion coefficients of molecular CO_2 (Dco_2) and bicarbonate ion (D_{HCO_3}) were separately determined by utilizing the Pco_2 dependence of α'. D_{HCO_3} was found to be reduced hyperbolically with increasing Hb concentration, whereas Dco_2 decreased exponentially. The Dco_2 and D_{HCO_3} values at 100 % hemolysate ([Hb] = 1) were 0.34×10^{-5} and 0.14×10^{-5} cm^2/sec, respectively.

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DERIVATION OF THEORETICAL EQUATIONS OF THE CO_2 DISSOCIATION CURVE IN MAN. MOCHIZUKI, M., TAZAWA, H., and KAGAWA, T. Dept. of Physiology, Yamagata University, School of Medicine, 990-23 Yamagata.

The CO_2 dissociation curve of oxygenated blood was calculated from the simultaneous Henderson-Hasselbalch equations in the red cell and plasma and another equation relating the intracellular pH change to the extracellular pH change. In order to accomplish the above calculation the water shift due to the Pco_2 change was measured in tonometered blood. The water concentrations in the red cell and plasma were given by the functions of the change in hematocrit. The hematocrit value of oxygenated blood was linearly correlated to pH with a factor of - 0.037. The buffer value of hemoglobin which was used for evaluating the bicarbonate change from the pH change was estimated from the curvature of the dissociation curve. It showed a close relationship with the blood CO_2 content at 40 Torr Pco_2. Using the above parameters together with the plasma buffer value of 7.5 mM/l,plasma·pH, the CO_2 dissociation curve was calculated. The blood and plasma CO_2 contents measured at four different Pco_2's were compared with the ones calculated by use of the intra- and extracellular bicarbonate contents measured at 42 Torr Pco_2 in 7 normal subjects. The measured and calculated CO_2 contents coincided fairly well with each other, suggesting the validity of the theoretical equations.

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CORRELATION OF BLOOD OXYGEN AFFINITY WITH TISSUE OXYGEN CONSUMPTION. I. SKELETAL MUSCLE AT REST. KOHZUKI, H., ENOKI, Y. and NAGANO, R.* 2nd Dept. of Physiology, Nara Medical University, Kashihara, Nara 634

This study was intended to clarify quantitative relationship between the tissue oxygen consumption and the oxygen affinity of perfusing blood as expressed by P_{50}, the oxygen tension for half-oxygenation of blood at pH 7.40, P_{CO_2} 40 torr and 37°C. By incubation of canine blood with bisulfite or phosphoenolpyruvate in an acid medium, the oxygen affinity was either elevated (P_{50}=25.2±3.4 torr) or lowered (P_{50}=44.5± 4.8 torr) as compared with the normal level (P_{50}=33.9±3.5 torr). These changes were shown to be induced by alteration in the red cell organic phosphates, mainly 2,3-diphosphoglycerate. Resting canine gracilis muscles in situ were regionally perfused with these bloods of different oxygen affinities at a constant flow rate per unit muscle weight and their influences on the oxygen consumption were determined. In normoxaemic perfusions (PaO_2=123.9±23.0 torr), no correlation was found between the oxygen affinities and the oxygen consumptions. In hypoxaemic conditions (PaO_2=58.6±9.4 torr), the oxygen consumptions were significantly decreased when perfused with the blood of lower oxygen affinity.

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FLUCTUATION OF VENTILATORY RESPONSE TO TRANSIENT HYPOXIA DURING THE MENSTRUAL CYCLE. TAKANO, N. Physiol. Lab., Dept. of Sch. Hlth., Fac. of Educ., Kanazawa Univ., Marunouchi 1-1, Kanazawa 920

Ventilatory response to hypoxia was studied on 9 healthy women at the intervals of 3-4 days throughout the menstrual cycle. A transient hypoxic test was applied, comprising 5-6 runs of a breathing sequence of air, N_2 (2-8 breaths in each run) and air. The resting values of respiratory parameters to be studied were obtained during the air breathing preceding the N_2. A maximum $\dot{V}_E$ was plotted against a minimum S_{aO_2} in each run, produced by the N_2 breathing, and the slope ($\Delta\dot{V}_E/\Delta S_{aO_2}$) of a linear regression calculated from all data in 5-6 runs was defined as the hypoxic sensitivity. Relative to the follicular phase, the following changes were observed during the luteal phase. (1) The hypoxic sensitivity was increased by 28%. (2) An 1.8-mmHg fall in resting P_{ACO_2} was seen even under the condition of no increase in resting $\dot{V}_E$, this being possibly due to a decrease in the physiological dead space. (3) The greater the fall in P_{ACO_2}, the greater the increase in hypoxic sensitivity, suggesting that the augmented hypoxic response in the peripheral chemoreceptor-brainstem system is elicited at the site of CNS but not at the receptor site.

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EFFECTS OF INTERMITTENT HYPOXIC EXPOSURES ON VENTILATORY RESPONSE DURING SUBMAXIMAL EXERCISE. MIYAMURA, M., SHIMAOKA, M., MATSUI, H., MORI, S. and TAKABAYASHI, A. Research Center of Health, Physical Fitness and Sports, and Institute of Environmental Medicine, Nagoya University, Chikusa-ku, Nagoya 464.

The present study was undertaken to examine the effects of intermittent hypoxic exposures on ventilatory response during rest and submaximal exercise. The subjects were 6 healthy male students aged from 19 to 21 years. The subjects were exposed for about 2 hours daily at barometric pressure corresponding to altitude of 4,500 m for consecutive 6 days. Maximum oxygen uptake, maximum ventilation , maximum heart rate, hypercapnic ventilatory drive at rest, hypoxic ventilatory drive during submaximal exercise were dertermined before and after intermittent hypoxic exposure for 6 days. Furthermore, oxygen uptake and pulmonary ventilation at rest and during submaximal bicycle exercise were measured daily at sea level and 4,500 m. It was found that there are no significant difference in the maximum oxygen uptake, hypercapnic drive at rest and hypoxic drive during submaximal exercise at sea level. However, oxygen uptake and pulmonary ventilation during submaximal exercise at 4,500 m lowered significantly on and after 3 day from first day, while it was not changed at sea level.

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EFFECTS OF HYPOXIA ON THE DEFORMABILITY OF RED BLOOD CELLS OF RAINBOW TROUT (SALMO GAIRDNERI). KIKUCHI, Y., KOYAMA, T. and HUGHES, G. M.* Div. of Physiol., Res. Inst. of Appl. Electr., Hokkaido University, Sapporo 060, *Res. Unit for Comp. Animal Resp., Bristol University, Woodland Road, Bristol BS8 1UG, England

The blood fluidity in microvessels is reduced with increment in the hematocrit value. This relationship will become especially important in animals exposed to hypoxia since a considerable increase in the hematocrit is induced by anoxic stresses. The present study examined microrheological properties of blood in relation to changes in the oxygen tension using rainbow trout. Blood samples were taken via arterial cannulae from fishes under the following conditions; a) normoxic in air-saturated water, b) hypoxic in nitrogen gas-bubbled water and c) asphyxic under anesthesia. In spite of elevated hematocrit values the blood samples b) and c) passed through micropores of 8 μm diameter much quicker than the samples a). A marked improvement was found in the deformability of red blood cells. Such changes were unobservable when the normoxic blood samples a) were equilibrated with a low oxygen gas in vitro. Although no correlation was found between the red cell deformability and the blood lactate and ATP levels, some other factors in plasma produced in vivo were suggested to be responsible for the marked improvements in the red cell deformability and hence in the blood fluidity in capillary vessels.

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ANALYSIS OF ISOCAPNIC HYPERPNEA USING ARTIFICIAL LUNG. TAKAHASHI, E.*, YAMAMOTO, K.* and MIKAMI, T. Biomed. Controls, Res. Inst. Appl. Elect., Hokkaido Univ.

Isocapnic hyperpnea is known as a response to mild exercise and venous CO2 loading, however, the mechanism of this phenomenon is not clear. To explain isocapnic hyperpnea, the feedforward controller which responds to the small oscillation in Paco2 synchronizing with the respiratory cycle was introduced. The feasibility of this hypothesis was evaluated through the animal experiment and computer simulation study. The dog was set on veno-venous bypass with a hollow fiber artificial lung, and tidal volume(V_T), respiratory frequency(f) and carbon dioxide production rate($\dot{V}co2$) were monitored. Small oscillation in Paco2(ΔPaco2) was calculated from the oscillation in pH(ΔpH) which was measured from the ISFET pH sensor inserted in the common carotid artery. The response of ΔPaco2 to the changes in V_T, f and CO2 loading/unloading were evaluated. ΔPaco2 was linear to the changes in $\dot{V}co2$. Paco2 was insensitive to the changes in V_T while ΔpH was dependent on V_T. ΔPaco2 and ΔpH were inversly proportional to f. These results demonstrate the feasibility of the model which combines $\dot{V}_E$ closely with $\dot{V}co2$ via ΔPaco2.

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DYNAMIC RESPONSES OF RESPIRATORY AND CIRCULATORY SYSTEMS TO EXERCISE STIMULI OF DIFFERENT PATTERNS. MIYAMOTO,Y. and MIKAMI,T. Dept. of Bio-Med.Control, Res.Inst.Appl.Elec., Hokkaido Univ., Sapporo 060.

Dynamic responses of ventilation, cardiac output and gas exchange characteristics of young healthy men to positive and negative bicycle exercise were studied. Cardiac variables were determined by an automated impedance cardiograph with a frequency of once per 10 sec. Respiratory and metabolic variables were also determined breath-by-breath. Harmonic analysis of positive work response revealed that $\dot{V}_E$,$\dot{V}O_2$,$\dot{V}CO_2$ and $\dot{Q}$ kinetics may be represented by a second-order transfer function consists of a slow and a fast components. There was a close correlation between $\dot{V}_E$ and $\dot{V}CO_2$ responses to positive work. It was found that $\dot{V}_E$ in response to a work with higher pedaling rate was slightly larger than that to a lower pedaling work although both the work loads were kept to be equal. Both $\dot{V}_E$ and $\dot{Q}$ increased in response to a negative constant work immediately after its onset and maintained the higher levels during the whole period of exercise. $\dot{V}O_2$ and $\dot{V}CO_2$ increased transiently at the begining but fell soon to respective resting level. End-tidal pCO_2 fell below its resting level. Thus, the correlation between $\dot{V}CO_2$ and $\dot{V}_E$ was failed to observe in the responses to negative work. These results lead to the conclusion that there is a neurogenic drive beside humoral stimuli to the respiratory center to augment $\dot{V}_E$ during exercise though its contribution to the total response is small.

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EFFECT OF CO_2 AND HCO_3^- ON SYNAPTIC TRANSMISSION IN THE RAT SUPERIOR CERVICAL GANGLION IN VITRO. FUKUDA, Y. Dept. of Physiology, School of Medicine, Chiba University, Inohana, Chiba 280

The mechanism of actions on the synaptic transmission of CO_2 or H^+, an important chemical factor for stimulating respiration, was studied on the sympathetic superior cervical ganglion of the rat in vitro. Supramaximal stimulation of preganglionic fibers with a single volley (0.3-0.5Hz) elicited initial spikes + N wave (fast EPSP), slow P wave (s-IPSP) and slow LN wave (s-EPSP) in the surface recording from the ganglion. Actions of CO_2 and HCO_3^- on spikes + N wave were examined. Decrease in pH of superfusing solution with high Pco_2 at a normal [HCO_3^-] slightly suppressed the N wave, whereas the pH reduction by low [HCO_3^-] at a normal Pco_2 induced a large decrease in N wave. Increase in N wave due to alkaline solution was much larger in high [HCO_3^-] (normal Pco_2) than in low Pco_2 (normal [HCO_3^-]) condition. When Pco_2 and [HCO_3^-] were simultaneously increased so that the extracellular fluid (ECF) pH remained unchanged, magnitude of N wave was greatly increased. Simultaneous reduction in Pco_2 and [HCO_3^-] (normal pH) decreased the N wave activity. These effects of changing Pco_2 and [HCO_3^-] on synaptic N wave activity were neither correlated with the ECF [H^+] nor with Pco_2, but they were well related to the ECF [HCO_3^-].

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EFFECTS OF SITS ON THE ACID-BASE AND ELECTROLYTE STATUS DURING ACUTE HYPERCAPNIA IN SPLENECTOMIZED DOGS. HATA, N., AKIYAMA, S. and HONDA, Y. Dept. of Physiology, Chiba University School of Medicine, Chiba 280

Effects of CO_2 ($12\%CO_2$-$30\%O_2$) inhalation on the acid-base and electrolyte status in blood and lymph were studied in SITS-treated (25mg/kg and 43.4mg/kg, iv) and untreated splenectomized dogs. The in vivo buffer slopes of arterial and mixed venous bloods were significantly higher in SITS-treated (43.4mg/kg) group (n=8) than in untreated one (n=18, $P<0.01$). Increments of plasma bicarbonate concentration were also higher in SITS-treated (43.4mg/kg) group (n=8) than in untreated one (n=18). Decrements of chloride concentration were slightly larger in SITS-treated groups (n=8 and n=3) than in untreated one (n=8). On the other hand, in vitro buffer slopes of the blood collected at 2 hours after SITS injection were decreased by 1.1 and 2.4 sl. ($P<0.05$) from the before SITS values in both SITS-treated (25mg/kg and 43.4mg/kg) groups, respectively. In the SITS-treated groups, nonextracellular buffering may have more contributed to increasing the value of the in vivo slope, because the ECF bicarbonate was considerably increased whereas in vitro buffering capacity of the blood was decreased. Higher ECF bicarbonate increments by SITS-treatment may have resulted from the inhibition of net bicarbonate influx into the tissues.

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PHYSIOLOGICAL RESPONSES TO $PICO_2$ (0.08 bar) UNDER HYPERBARIC ENVIRONMENT IN CATS. SEKI, K., MIZUSHIMA, Y., TAYA, Y., IWASAKI, M., KUWABARA, N. and TANABE, M. Japan Marine Science and Technology Center,Natsushima-cho,Yokosuka 237

It is well known what is the cardiovascular response to high $PICO_2$ under atmospheric environment in many mammals. However, those under hyperbaric environment and high pressure inert gases are still unknown. In the present study we continuously observed the physiological responses to $PICO_2$ (0.08 bar) especially the awareness of cats under 11 ATA $He-O_2$,N_2-O_2 hyperbaric environment. The CO_2 toxity was not observed under $He-O_2$ environment,which is inconsistent to the past studies. The decreasing of the sensitivity to CO_2 toxity was observed under N_2-O_2 environment.

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ALTERATION OF fH AND SENSITIVITY TO CO_2 ($P_{0.1}$) DURING BREATH-HOLD DIVING IN JAQUES MAYOL. MIZUSHIMA,Y*,SEKI,K*,NAKAYAMA,H**,TAYA,Y*,IWASAKI,M*, KUWABARA,N* *Japan Marine Science and Technology Center.2-15.Natsushima-cho Yokosuka**School of Med. Univ. of Occupational and Environmental Heahlth.Kitakyushu.

The purpose of this study is to compare the respiratory function among three divers. One is Jaques Mayol, a famous breath-hold diver in France, and another two divers are male and famale professional ones called "Ama" in Japan. The Maximum underwater breath-hold time of Mayol was 4 min. and 9 sec, while that of male Ama was 2 min. and 9 sec. It appeared remarkable bradycardia in Mayol during breath-hold diving. It was revealed from $P_{0.1}$ recording that the sensitivity to CO_2 of male Ama was lower than that of Mayol whose sensitivity was similar to that of ordinary Japanese adult. The vital capacity showed similar pattern. The exact machanism of extraordinary long breath-holding time of Mayol has been still unknown. However, if the machanism would be solved in the future studies and if longer breath-hold diving in the ordinary divers would be realized with a special training , man would be able to have a wide behavioural range under water.

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RESPIRATORY RESPONSES INDUCED BY ELECTRICAL AND CHEMICAL STIMULATION OF MUSCULAR THIN-FIBER AFFERENTS IN THE CAT. TADAKI, E. and KUMAZAWA, T. Kinjo Gakuin University, 1st Dept., Res. Inst. of Environ. Med., Nagoya University, Chikusa-ku, Nagoya 464

In anesthetized, vagotomized, carotid sinus deafferented, paralyzed, and artificially ventilated cats, respiratory responses to both electrical stimulation of the muscle nerve and chemical stimulation of muscular polymodal receptors by means of intra-arterial injection of NaCl solution were studied by recording phrenic nervous discharges. Electrical stimulation of muscular afferents caused 3 types of respiratory responses: 1) with a stimulation above A-δ fiber threshold, a suppressive phase was observed immediately after the start of stimulation and disappeared by cessation of stimulation (initial suppression). 2) intensity-dependent facilitation of respiratory outputs was observed during the period of stimulation. 3) with C fiber stimulation range, respiratory outputs decreased significantly below the pre-stimulus value within several minutes after the cessation of the stimulation (after suppression). Chemical stimulation of polymodal receptors caused concentration-dependent respiratory facilitation during stimulating period and "after suppression" at high concentration as well, however, failed to evoke "initial suppression" at any concetration. Naloxone, the opiate antagonist, reversed the "after suppression" caused by both types stimulation but did not affect the "initial suppression".

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EFFECT OF INJECTION OF HIGH CO_2 BLOOD INTO THE ARTERIES OF THE VENTRAL SURFACE OF THE MEDULLA ON THE PHRENIC NERVE ACTIVITY. KUWANA, S. and NATSUI, T. Dept. of Physiol., Teikyo Univ. School of Med., Itabashi-ku, Tokyo.

Integrated phrenic nerve discharges were recorded as an output of respiratory activity in anesthetized, vagotomized and artificially ventilated cats. One ml. of blood, which was equilibrated with a gas mixture of 80% CO_2 and 20% O_2 at 37°C, was injected into the vertebral artery (VA) or the arteries of the ventral surface of the medulla. The injection into the VA produced an increase in the phrenic nerve activity (VA injection response). VA injection response was maintained even if the basilar artery was occluded at the level between the medulla and the pons, while the injection into the basilar artery toward a rostrum from the occluded region did not increase the phrenic nerve activity. VA injection response was mostly disappeared by the bilateral occulusions of the anterior inferior cerebellar arteries (AICA) that are branches from the basilar artery. Furthermore, an increase in the phrenic nerve activity was produced not only by the injection into the AICA but also by the bilateral occulusions of the AICA alone. These results indicate that the central chemosensitive areas in respiratory regulatory system are mainly perfused by the blood of the AICA.

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LOCALIZATION AND FUNCTION OF RESPIRATORY NEURONS IN THE UPPER CERVICAL CORD OF THE CAT. AOKI, M., KASABA, T. and KUROSAWA, Y. Dept. of Neurophysiology, Sapporo Medical College, Chuoku, Sapporo 060.

We have recently demonstrated that respiratory neuron activities could be recorded extracellurarly and intracellularly as well within the upper cervical cord in lightly anesthetized or decerebrated cats. Most of the units showed the inspiratory neuron discharges in synchrony with the efferent phrenic nerve activities. The recording sites were histologically located in the intermediate zone of the spinal gray matter of C1-C2 segments. We examined if any cervical respiratory neurons send descending axonal projections and if they receive synaptic inputs from the medullary respiratory nuclei. 1) Most of the neurons (92%) were antidromically activated by the stimuli delivered to the ipsilateral C5-C6 ventral funiculus. Antidromic activation was confirmed by collision test. The antidromic conduction velocities ranged from 25-45 m/s. 2) In other experiments, unit discharges of cervical and medullary respiratory neurons were simultaneously recorded using tungsten microelectrodes. Then the electrode inserted to the medulla was switched to a stimulating electrode and the Nucl.retroambigualis was stimulated. In approximately 93% of the neurons, orthodromic action potentials were elicited contralaterally with the latencies of 2.0-8.0 (mean 4.0) msec, while in 54% of the neurons, ipsilaterally with the latencies of 2.0-7.0 (mean 4.7) msec.

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DIFFERENTIAL EFFECTS OF THIAMYLAL ON BULBAR RESPIRATORY NEURONS WITH REFERENCE TO STABILITY OF THEIR SPONTANEOUS UNITARY DISCHARGES. KIGUCHI, Y., TAKANO, K., KIMURA, N. & HUKUHARA, T., Jr., Dept. of Pharmacol. II, Jikei Univ. Sch. of Med., Minato-ku, Tokyo 105.

The relation of the grade of changes in the respiratory unit activity caused by thiamylal to the degree of stability of spontaneous unitary discharges of bulbar respiratory neurons in the rabbit was studied. Respiratory units discharging unstably ceased to fire by smaller doses (2-8 mg/kg i.v.) when the phrenic burst discharges persisted after thiamylal. On the other hand, stable units continued to discharge during the phase in which burst discharges of the phrenic nerve activity were abolished by thiamylal. The mean value of correlation coefficient of autocorrelation (CC) for unstable respiratory neurons in the control before the drug was significantly smaller than that of stable respiratory neurons. The control mean value of coefficient of variation (CV) for the volley period in the former group was significantly larger than that in the later group. The degree of drug-induced decreasing changes in the mean spike frequency in volley significantly correlated to CCs of respiratory units before the drug. These findings indicate that stable respiratory neurons are tolerant to the inhibitory action of thiamylal while unstable ones are markedly influenced by thiamylal. These results suggest that respiratory neurons showing different degree of stability may play different functional role in the bulbar mechanisms for the respiratory rhythmogenesis.

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SYNCHRONIZATION OF INSPIRATORY AND EXPIRATORY ACTIVITIES INDUCED BY PHARMACOLOGICAL AGENTS. SHIMADA, K., MIYAOKA, Y., IKUNO, H., SATO, S.*, and TAKAHASHI, Y.* Dept. of Physiol., Niigata Univ., Sch. of Dent., Niigata.

Discharge pattern of respiratory neuron is changeable with effects of pharmacological agents. In previous study we reported a shift of expiratory activity and sychronizing excitation of the phrenic nerve and the internal intercostal muscle activities. In present study we investigated similar synchronization of phrenic nerve expiratory neural activities in paralyzed animals. Rats, rabbits, and cats were anaesthetized with α-chloralose urethan and vagotomized and paralyzed. Expiratory activity was recorded from the intercostal and expiratory branch of the pharyngeal constrictor nerve. Two types of synchronization of inspiratory and expiratory activities were induced by i.v. application of strychnine and/or picrotoxin. The first type of synchronization was so-called strychnine tetanus in which respiratory rhythmicity was almost disappeared. In second type the phase of expiratory neural activity shifted gradually into the phase of inspiraty activity and ultimately these two activities synchronized completely.

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DEEP RESPIRATION FACILITATES VIBRATION-INDUCED FLEXION REFLEX (VFR) AND SKIN VIBRATION FACILITATES RESPIRATION. HOMMA, I., OBATA, T., SAKAI, T. Dept. of Physiol. The Jikei Univ. School of Med. Tokyo

The effects of forced or deep respiration on exteroceptive vibration-induced flextion reflex (VFR) and the effects of skin vibration on ventilation were studied in healthy man. The experiments were performed on 12 normal adult volunteers. A middle finger of each subject was unsupported and the finger tip was connected to a strain gauge with a rubber band to measure finger flexion force. Respiratory flow volume was measured by a hot wire respiratiry flow meter mounted on a mouth piece. A vibration was applied on the volar side of the middle finger to induce the VFR. Vibration was applied for 20 sec during forced expiration or during rebreathing. VFR contraction increased during deep breath obtained by forced expiration or rebreathing. Ventilations were also increased due to either shortning of inspiratory time or increasing tidal volume by vibration of the skin.

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ROLE OF THE STERNOHYOID MUSCLE IN THE FROG'S RESPIRATION. SAKAKIBARA, Y., Dept. of Physiology, School of Medicine, Chiba University, Inohana Chiba 280

The frog buccal pump system serves to inhale a fresh air into the mouth(buccal inhalation)during the first phase of lung respiration cycle. It is attributed mainly to the sternohyoid muscle. The present study revealed that the electroneurogram of the nerve innervating this muscle(ENGsh) appeared not only in the buccal inhalation phase, but also in the succeeding phase, lung expiration, in the bullfrog. In the frog with glottis occluded, lung pressure(LP) elevation was clearly demonstrated in the lung expiration phase. The magnitude of the LP change in this phase varied proportionally to ENGsh and attained as high as 1.5 cmH_2O in response to severe asphyxic drives. In the same condition, electrical stimulation of this muscle elevated LP by the same magnitude as mentioned above. On the other hand buccal pressure reduced by as much as 3 cmH_2O while nostrils concealed. ENGsh corresponding to buccal inhalation increased in the process of lung inflation sequences induced by hypoxic stimulation. On the contrary ENGsh corresponding to lung expiration did not increase but severely diminished in the same process. In the deflation process followed from inflation, either phasic activity turned back to each control level. These results supported the hypothesis that the sternohyoid muscle acts not only to enlarge the buccal cavity, but also to compress the lung, and that in the lung expiration phase it fascilitates the expiratory flow.

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POSSIBLE ROLES OF 5 -ANDROSTANE COMPOUNDS IN DEVELOPMENT OF MURINE SALIVARY GLAND. KATSUKAWA, H. and FUNAKOSHI, M. Dept. of Oral Physiology, Gifu College of Dentistry, Motosu, Gifu 501-02

Action of circulating androgens on the submaxillary gland(SMG) was studied in infant mice by examining responses of an esteroproteolytic enzyme(tamase) in female gland to exogeneous androgens and by measurement of the activity of steroid-metabolizing enzymes involved in androgen metabolism. Testosterone(T), 5α-dihydrotestosterone(DHT) and 5α-androstane-3α,17β-diol(3α-diol) produced a siginificant increase in tamase activity above control values. DHT and 3α-diol elevated the enzyme activity to an approximately equal level whereas the potency of T was relatively low compared with that of these two androgens. Long term exposure to androgen, T or 3α-diol(3 μg/mouse), accelerated androgen responses of tamase. A relative activity of 5α-reductase increased with age in males and was relatively constant in females when the activity of 3α-hydroxysteroid dehydrogenase was assigned a value of 1.0. The results suggest that during prepubertal period DHT and 3α-diol have cummulative effects on androgen responsiveness of male SMG whereas development of androgen responsiveness in female SMG is regulated by 3α-diol.

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THE EFFECTS OF UNAVAILABLE CARBOHYDRATE ON ENLARGEMENT OF THE CECUM AND COLON IN RAT. OKU, T. and HOSOYA, N. Dept. of Nutrition, Faculty of Medicine, Univ. of Tokyo, Bunkyo, Tokyo 113, Japan.

This study was designed to investigate the changes in structure and/or function of the cecum and colon during prolonged consumption of unavailable carbohydrate and the recovery of increased tissues after ceasing unavailable carbohydrate consumption. When rats were raised on a diet containing 20% glucomannan, pectin and pullulan for 8 weeks, the wet weight of the cecum significantly increased compared with that of the control rat fed an unavailable carbohydrate free diet. The weight increases in the colon were detected in rats fed 20% cellulose diet. Measurement of DNA, RNA and protein in the cecum and colon mucosa demonstrated that tissue increase caused by unavailable carbohydrate intake for 4 weeks resulted from an increase in cell number and size, that is hyperplasia. (Na+K)ATPase activity in the cecum and colon increased significantly in tissue with hyperplasia. The intake for 4 weeks was rapidly brought back to the control level within a week after the switching to a unavailable carbohydrate free diet. Also, (Na+K)ATPase activity was decreased to that of control animals.

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A HYPOTHESIS OF SYMBIOTIC ORGANISMS SIMULATING FUNCTIONS OF THE DIGESTIVE-ABSORPTIVE-SECRETORY SYSTEM (4). TSUKEDA, K. Dept.of Physiol., Fac.of Med., Univ.of Tokyo, Tokyo

Salivary gland:Bacillus subtilis (B) contains an amylase. Stomach:HCl may be formed by strongly acidic sulfer bacteria (S) and acid-resistive halobacteria (N). By symbiosis of S with parietal cells and N with main cells, H^+ from the former and Cl^- released from the latter may form HCl in the presence of carbonic anhydrase. The ancester of this enzyme may be purple bacteria, Rhodopseudomonas (R). Pancreas:Mold fungi in pancreatic juice contain various enzymes for glycolysis and proteolysis. Some Pseudomonas contain lipase or phosphorylase. B may also have symbiosed. Liver:Yeast and Mitochondria, having enzymes for glycogenogenesis, oxidative phosphorylation and proteolysis, synthesize urea and NH_3. Streptomyces produces ribosomes and synthesizes ATP. Amoebas have a phagocytotic action. Bacillus polymyxa disposes red corpuscles, produces bile acids, and contains a coagulation substance of blood. These organisms grouped together and differentiated into the liver. R may seek for dark aerobic liver and repeatedly circulate between this organ and photoanaerobic tissues. Intestine:Enterobacters have membrane proteins, fermented lactose, and require Na^+, Mg^{++}, Ca^{++}. Each Lactobacillus needs one amino acids. Escherichia coli likes bile acids. Colon:Clostridium butyricum requires methionine and Na^+. Hydrogenomonas utilizes H_2O. As stated above, each procaryotic organism has one main action and symbioses with its favorite tissue. Presumably, procaryotic organisms and eucaryotic living-beings have differentiated through continuous cohabitation.

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STUDIES ON PENETRATION PHENOMENA THROUGH INTESTINAL MEMBRANE — relationship between intestinal penetration and line of magnetic force (2) NAKANO,S.,MIYOSHI,M.,NAGAMI,K., YOSHIOKA,T. Dept. of Physiol., The Tokai Univ. Sch. of Med.,259-11 Isehara, Kanagawa.

In previous studies, by use our devised conventional circulating apparatus, we have investigated a series of experiments on penetration through intestinal membrane of insulin as activepolypeptides and L-tryptophane as well as activetransport of glucose to penetration efficiency. This apparatus can measure penetration phenomena of these substance, through the intestinal wall by automatic determining to the ultra-violet absorbance. However the conventional apparatus wich uses everted intestine can define intestine length but cannot define its surface area accurately. To cope with this difficulty, we have devised a new apparatus wich is able to define intestine surface area, as well as to examine the effect of magnetic force on penetration, applying line of magnetic force from mucosal side to serosal side or in reverse direction. The obtained experimental results with this new apparatus are as follows: 1)Penetration through intestinal wall of substances was measured with higer accuracy than by conventional apparatus. 2)When intestine was irradiated from mucosal side to serosal side by 6000 Oe magnetic force, insulin and L-tryptophane penetration showed tendency increased, but glucose penetration was slight inhibited. 3) On the contrary, when irradiated from serosal side to mucosal side, the penetration of these substances were obviously inhibited. 4) Change of transmural potential difference showed a slight decline by penetration of these substances through intestinal wall.

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HORMONAL CORRELATES IN THE ETHOSECRETOGRAM IN RATS.
SHIRAISHI, I., HONMA, K., HONMA, S. and HIROSHIGE, T. Dept. of Physiology, Hokkaido University School of Medicine, Kita-ku, Sapporo 060

Previously we showed (J. Physiol. Soc. Japan 44: 518, 1980) that bursts of feeding activity were closely correlated with those of plasma corticosterone secretion in freely moving intact rats (ethosecretogram). In the present study we examined changes of plasma insulin level in response to feeding in intact rats and the effect of bilateral adrenalectomy on it. Plasma insulin was determined by RIA, corticosterone by a competitive protein-binding assay and glucose by an oxidase method. The adrenalectomized rats were maintained on drinking saline. Both intact and adrenalectomized rats were fasted for 24 or 48 hours and allowed to feed for 2 hours. On feeding, plasma insulin level rose less in adrenalectomized than in intact rats. Next, feeding was restricted to 2 hours a day for 2 weeks. At the end of this restricted feeding, plasma glucose and insulin were again followed. We found that the incremental response of plasma insulin was much reduced in adrenalectomized rats, even though there was no essential difference in the plasma glucose elevation. It is surmised that increments of plasma corticosterone secretion on feeding, or even prior to feeding, are related, directly or indirectly, to a concomitant change in plasma insulin secretion, resulting in a homeostatic regulation of energy substrate level.

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PRE-FEEDING CORTICOSTERONE PEAK UNDER RESTRICTED FEEDING: MANIFESTATION OF THE HORMONE PEAK AND TIME-INTERVAL MEASUREMENT.
HONMA, K., HONMA, S. and HIROSHIGE, T. Department of Physiology, Hokkaido University School of Medicine, Sapporo 060

Effects of meal feeding on plasma corticosterone levels were examined in rats under various feeding schedules. Pre-feeding corticosterone peak was observed when meal was restricted to 2 h at a fixed time of day but it was not detected when food availability was extended to 6 h. The amount of food intake under the latter condition was comparable to that in a whole day under ad lib. feeding. Each meal has a bidirectional effect on the hormone level, namely postprandial elevation of the basal level and postprandial reduction of the high pre-feeding level. When rats were fed with feeding intervals of 22, 24 and 26 h, the hormone peak showed phase angle differences of 30 min, 2.5 h and 4.5 h, respectively. When the interval was extended to 30 h, a hormone peak appeared not immediately but several hours prior to meal, and when it was shortened to 18 h, the peak disappeared but was revealed when regular meal was omitted; the interval between a preceding meal and the hormone peak was similarly 18-20 h in both cases. It is concluded that the amount of food intake is critical for the development of the pre-feeding corticosterone peak and the hormone pattern under restricted feeding is determined primarily by three factors; a basal circadian rhythm, a meal-associated process which measures the time-interval of 20 h and a bidirectional effect of meal.

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THE REPRODUCTIVE RHYTHM AND CIRCADIAN RHYTHM IN AGED FEMALE RATS.
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Aged female rats show a persistent estrus(PE), a pseudopregnant(PSP) or a persistent diestrus(PD). In this study, the age related changes in circadian rhythmicity of the sleep in old female rats and the central mechanism of the aging process by comparing with the sleep rhythm of several aging models were investigated. The slow wave sleep(SWS) and paradoxical sleep(PS) in old PE rats showed a single peak during daytime in 9/15 PE rats, but 6/15 PE rats showed ultradian PS rhythm. The former resembled the 6-OHDA treated rats or the rats bearing the ascending noradrenergic nerve transection which showed the postsynaptic super-sensitivity of noradrenergic receptor, the latter resembled the intra suprachiasmatic(SCN) PCPA injected rats which showed the decreased hypothalamic amins contents (Walker et al, 1980). Old PSP rats showed the semicircadian PS rhythm with higher night peak like as the rats lesioned medial preoptic area. Old PD rats indicated the ultradian SWS and PS rhythm like as the rats lesioned large hypothalamic areas including SCN. The exposure of constant dark or light caused the reduction of rhythm amplitude of SWS in old rats. The estradiol(E2) treatment in ovariectomized old rats caused the delay of E2 induced night PS reduction which correlated the E2 positive feedback. From these results, it is supposed that the sleep rhythm reflects the age related changes in brain amins and the aging affects the steroids feedback mechanism and central time-measuring mechanism.

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LH SECRETORY RHYTHM IN THE PREPUBERTAL FEMALE RAT. KIMURA, F. and TSAI, C.-W.*
2nd Department of Physiology, Yokohama City University School of Medicine, 2-33 Urafune-cho, Minami-ku, Yokohama 232

In order to search for the ground underlying the ovulatory secretion of LH that begins definitely at 14.00 h on proestrus, detailed secretory profile of LH secretion in the 29-31 days old female rat was studied by serial blood sampling through an intracardiac cannula at 15-min intervals for each of the 6-h period beginning at 12.00 and 00.00 h. By analysis of the power spectrum and the least squares method, the time sereis of LH concentrations which were measured by RIA were found to have 3.25 ± 0.17 (mean ± SE, n=28) h for the period 12.00-18.00 h and 3.48 ± 0.26 (mean ± SE, n=15)h for the epriod 00.00-06.00 h. The time series of the number of LH peak, whose time was calculated based on the acrophase estimated, revealed a 2.87-h periodicity having peaks at 12.00, 14.52 and 17.44 h, and a 4.60-h periodicity having peaks at 00.00 and 04.36 h, respectively. Since the amplitudes of LH secretory episodes around 12.00 h were markedly small compared with those around 15.00 and 18.00 h, it was speculated that secretory episodes peaking at 14.52 and 17.42 h, among episodes which occurred with ultradian rhythm of approximately 3-h periodicity, would develop, through certain modulation, into the proestrous secretory episode of LH which began at definite time; i.e., at 14.00 h.

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EFFECTS OF INTRAVENTRICULAR INFUSION OF ENDORPHIN AND NALOXONE ON PULSATILE LH RELEASE IN OVARIECTOMIZED RATS. KUBO, K., KIYOTA, Y. & TORII, M. Dept. of Physiol., Inst. of Constitutional Med., Kumamoto Univ., Kumamoto 862

Effects of prolonged infusion of β-endorphin (EP) or naloxone (NLX) in a saline vehicle at a rate of 20 µl/h into the third ventricle were studied in adult ovariectomized rats. Unanesthetized rats with right atrial cannulae were bled continuously at a rate of 40 µl/5 min for 6 h including 1.5 h of preinfusion period and 2/3-2 h of infusion period. Blood samples were analysed for LH by RIA using killed *Staphylococcus aureus* as a separating agent. Infusion of saline had no effect on mean LH levels nor pulsatile pattern. Administration of EP (20 µg/h) resulted in a loss of pulsatile pattern and a decrease in blood LH to a lower level than mean nadir LH levels of preinfusion pulses. In contrast, infusion of NLX induced an increase in LH levels. The rise of LH began rapidly, and lasted for the entire infusion period at a dose of 10-20 µg/h but for a part of infusion period at a dose of 100-200 µg NLX/h. It was followed by a decrease in LH levels and intermittent LH discharges with low frequency and high amplitude. During the rising period, pulsatile pattern was lost and the mean LH levels were equivalent to mean peak LH levels of the preinfusion LH pulses. NLX can maintain the LH release to maximum level of pulsatile activities. Present data suggest that the hypothalamic EP and LH-RH neurones may constitute a negative feedback circuit consisting of a basic unit to generate pulsatile LH-RH activities.

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RESPONSES OF IDENTIFIED CENTRAL GRAY CELLS TO THE MICROIONTOPHORESIS OF LHRH AND ITS ANALOGS. SAKUMA, Y. Department of Physiology II, Niigata University School of Medicine, Niigata 951

Neurons in the central gray (CG) of the urethane-anesthetized female rat midbrain were classified by their responses to electrical stimulation of the medullary gigantocellular nucleus (NGc) and of the ventromedial hypothalamic nucleus (VMN), as well as by responses to somatosensory stimuli (pressure on the perineum). Effects of the iontophoresis of LHRH and its analogs were examined in 37 cells, which responded to at least one of the three stimuli. NGc stimulation induced transsynaptic (n=10) or antidromic (n=14) facilitation. VMN stimulation facilitated (n=7) or inhibited (n=1) the CG neuron. Somatosensory stimulation facilitated 30 of the cells. Except those driven antidromically from the NGc, almost a half of the cells in each group responded to LHRH; none of the antidromically driven CG neurons showed immediate response to LHRH. An LHRH analog, des-Gly^{10}-[D-Ala^{6}]-LHRH-EA, which has been shown to suppress lordosis reflex of the female rat, antagonized LHRH in the CG. It was concluded that LHRH acts in the CG on local neurons, on which hypothalamic and peripheral inputs converge.

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INFLUENCE OF ESTRADIOL BENZOATE ON SEX DIFFERENTIATION OF GONAD IN QUAIL EMBRYO AND POULT. II. YAMAMOTO, N. and ITO, Y. Dept. of Physiology, Gifu University, School of Medicine, Tsukasa-machi 40, Gifu 500.

Estradiol benzoate (0.02mg or 0.03mg) was injected into yolk of fertirized egg of genetic male quail (sw/+) on the early stage of incubation. Histologically, gonadgenesis of embryo and poult of quail was studied. Section profile in the largest area of both right and left gonad was measured as compared with that of control male. Area of the cortex of left testes on the treated group was about $3x10^{-2}mm^2$ on nine day embryo, about $5x10^{-2}mm^2$ on fifty day embryo and about $8x10^{-2}mm^2$ on seven day after hatching, and was increased over that of the control left testes (about $1.5x10^{-2}mm^2$ on nine day embryo, about $2x10^{-2}mm^2$ on fifty day embryo and about $3x10^{-2}mm^2$ on seven day after hatching). On the other hand, area of the right gonad in treated male was about $3.5x10^{-2}mm^2$ on nine day embryo and about $6.5x10^{-2}mm^2$ on fifty day embryo, and showed a great decrease against that of control right testes (about $5x10^{-2}mm^2$ on nine day embryo and about $12x10^{-2}mm^2$ on fifty day embryo). Such a change of relative area in the male gonad was similar to that seen in the female.

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ACETYLPOLYAMINES FOUND IN THE HAMSTER EPIDIDYMIS. MATSUZAKI, S. Dept. of Physiol., Inst. of Endocrinology, Gunma University, Maebashi 371

The hamster epididymis is quite unique in that it contains N^1-acetylspermidine, N^1-acetylspermine and homospermidine in addition to the common polyamines, putrescine, spermidine and spermine. All these compounds were found in highest concentrations in the distal caput epididymidis from photoperiodically-stimulated hamsters. Both the caput and the cauda were very active in acetylating spermidine and spermine; the rate of acetylation of the former was several times greater than that of the latter. In the rat epididymis only trace amounts of acetylated polyamines were detected, though the N^1-acetyltransferase activity was nearly as active as in the hamster epididymis.

Bilateral orchidectomy reduced the content of N^1-acetylspermidine and of putrescine, especially in the distal caput of the hamster. Treatment of the castrated animals with testosterone resulted in an increase in these polyamines. The content of other polyamines was changed only a little by these treatments. These results suggest that the formation of putrescine from spermidine via N^1-acetylspermidine in the epididymis is regulated by androgen.

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HETEROGENEITY OF IMMUNOREACTIVE GLUCAGON IN SYNCHRONIZED GLUCAGON-PRODUCING TUMOR CELLS. SAKAI, S., IMAGAWA, M., SAITO, H., AIZAWA, K., O'HATA, S., AKIYAMA, S.[1]*, KANAZAWA, M.[1] and SUGIMOTO, T.[1] Dept. of Physiol. & Int. Med.[1], Tokyo Med. Coll., Shinjuku-ku, Tokyo 160

We have established glucagon-producing tumor cell line (ITC-1) by subcutaneous inoculation of insulinoma(In 111) cells to Syrian hamsters. ITC-1 cells were grown as monolayers in RPMI-1640 medium supplemented with 10% newborn calf serum. Exponentially growing cells were synchroneized by treating double thymidine blocks. Immunoreactive glucagon(IRG) was assayed with C-terminal antibody(OAL-123). The cell cycle analysis was performed by a FACS-II fluorescence-activated cell sorter. Maximum IRG concentrations in the medium and in the cells were found at G_2-phase, and minimum concentrations at M-phase. Gel filtration of acid-EtOH extracts from synchronized ITC-1 cells on Bio Gel P-30 yielded four OAL-123 immunoreactive fractions($IRG^{>40000}$, IRG^{9000}, IRG^{3500}, IRG^{2000}). The elution profiles of four IRGs varied in stages of the cell cycle. Synchronized ITC-1 cells may be useful for studying the biosynthesis and processing of glucagon.

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REGULATION OF AMYLASE RELEASE BY CATECHOLAMINE IN RAT PAROTID SLICE. YOSHIMURA, K. NEZU, E. and YONEYAMA, T. Dept. of Physiology, Hokkaido University School of Dentistry, North 13, West 7, Kitaku, Sapporo, 060

Previously we have shown that an increase in amylase release by catecholamines is not always associated with a parallel change in the level of cyclic AMP or the activity of cyclic AMP-dependent protein kinase ; that is, a slight increase in cyclic AMP or the protein kinase activity is sufficient for the maximum stimulation in amylase release (Yoshimura et al. Jpn. J. Physiol. 32, 699-716,1982). However, amylase release stimulated by forskolin and isobutyl-methylxanthine was much lower than that induced by isoproterenol, although they significantly increased both the level of cyclic AMP and the activity of the protein kinase. These results suggest that even though amylase release by β-agonists is regulated by a cyclic AMP system, other factors or mechanism may participate in its regulation. In this connection, it is interesting that amylase release by α-agonists such as phenylephrine or methoxamine was markedly augumented by forscolin or isobutyl-methylxanthine, whereas the effect of other secretagogus was not. Similarly amylase release by methoxamine was increased by dibutyrylcyclic AMP. These results suggest that amylase release by α-agonists is regulated by the combined action of the cyclic AMP system and other factors or mechanism.

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EFFECTS OF ACUTE DIABETES ON AMYLASE SECRETORY CYCLE IN RAT PAROTID GLANDS. KURAHASHI, M., NAKAMURA, H. and INOMATA, K*. Dept. of Oral Physiol. and Physiol*., Higashi-Nippon-Gakuen Univ., School of Dent., Ishikari-Tobetsu, Hokkaido 061-02

Effects of acute diabetes on isoproterenol-induced amylase secretory cycle was investigated in rat parotid glands. In normal rats, a single injection of isoproterenol decreased the parotid soluble protein content and amylase activity within 2 hr. These parameters almost returned to the initial preinjection levels 24 hr after injection. The weight of parotid glands increased from 2 hr to 24 hr after injection. These changes are consistent with the initial amylase output from parotid glands and amylase resynthesis in parotid glands. In streptozotocin-induced diabetic rats, the initial decreases in parotid soluble protein content and amylase activity were smaller than those in control ones, and the recovery of these parameters was also smaller than that in control ones. The weight of parotid glands did not change anytime after injection. Isoproterenol increased the plasma insulin level in normal rats. In streptozotocin-induced diabetic rats, the plasma insulin levels was lower than that in normal rats, and this level did not change after isoproterenol injection. These results suggested that insulin is necessary for the presence of isoproterenol-induced amylase secretory cycle in rat parotid glands.

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THE NEURONAL ACTIVITIES IN THE LIMBIC SYSTEM DURING MALE COPULATORY BEHAVIOR IN RATS. HORIO,T., HANADA,M., SHIMURA,T. and SHIMOKOCHI,M. Dept. of Behav. Physiol., Fac. of Human Sciences, Osaka Univ., Suita-shi, Osaka-565

We recorded the multiple unit activity (MUA) in the medial preoptic area (MPO) and the amygdala (AMY) during male copulatory behavior in rats. From introduction of female to ejaculation, the background level of MUA in MPO increased about 20% above the level before introduction of female. Prior to each mount, the MUA suddenly increased toward it several times as much as the background level. After mount without intromission, the MUA decreased for few seconds below the background level. After mount with intromission, it decreased for several tens of seconds, but after mount with ejaculation, it remarkablly decreased for a few minutes below the background level. Although the MUA also in AMY increased prior to the initial several mounts, it returned gradually to the pre-mount background level toward ejaculation as well as sniffing behavior. The MUA in AMY was not so specific to male copulatory behavior as that in MPO.

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THE EFFECT OF TAIL PINCH UPON NEURONAL ACTIVITIES IN THE MEDIAL PREOPTIC AREA OF RATS. SHIMURA,T., HORIO,T. and SHIMOKOCHI,M. Dept. of Behav. Physiol., Fac. of Human Sciences, Osaka Univ., Suita-shi , Osaka-565

We found that sexually inactive male rats started to pursue female to copulate following brief tail pinch (TP), and the multiple unit activity in the medial preoptic area (MPO) increased concurrently with TP. Then, in order to identify MPO neurons which specifically relate to male copulatory behavior of rat, we recorded extracellular single unit activities of MPO and examined the effect of TP under light urethane anesthesia. In male rats, 41 out of 119 neurons (34.5%) responded to TP. 20 neurons (16.8%) were excitatory and 21 (17.6%) were inhibitory. Most of MPO neurons (57.1%), regardless of responsive or unresponsive to TP, had the low spontaneous firing rate (less than 7/sec). In female rats, the spontaneous firing rate of MPO neurons were almost the same distribution as male rats. However, responsive neurons to TP were infrequently found (5/33; 15.2%) compared with male. From these results, it was assumed that excitatory neurons to TP in male MPO might relate to sexual arousal of male rats.

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EFFECT OF THE INTRAVENTRICULAR ADMINISTRATION OF LEU- AND MET-ENKEPHALIN ON THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS IN DOGS. MATSUMOTO, I., MORIKAWA, T., TSUJIMOTO, Y. and AIKAWA, T. Dept. of Physiology, Nagasaki University school of Medicine, Nagasaki 852

The adrenocortical secretion in response to the intraventricular administration of Leu- and Met-enkephalin was examined in dogs. Leu- or Met-enkephalin (1 and 100 pmole/ml) was infused into the third ventricle at the rate of 15.6 µl/ml for 20 min under the pentobarbital anesthesia. The secretion rate of 11-Hydroxycorticosteroids (11-OHCS) from the adrenal vein cannulated on the day before experiment was measured as an index of ACTH secretion. Leu-enkephalin at both doses 1 and 100 pmole/ml produced a remarkable increase in 11-OHCS secretion rate with two peaks at 10 min and 120 min after the onset of infusion. Met-enkephalin at doses 1 pmole/ml and 100 pmole/ml induced a small 11-OHCS secretory response continuing from 5 to 90 min and from 60 to 150 min after the onset of infusion, respectively. Simultaneous intraventricular administration of 100 pmole/ml naloxone at the rate of 15.6 µl/min for 25 min almost completely abolished the increases in adrenocortical secretion induced by Leu- and Met-enkephalin (100 pmole/ml). Both enkephalins may play a role on the regulatory mechanism of the hypothalamo-pituitary-adrenal axis in dogs.

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EFFECTS OF THE HYPERTONIC STIMULUS ON THE ACTIVITY OF THE RECURRENT INHIBITION IN THE PARAVENTRICULAR-NEUROHYPOPHYSEAL COMPLEX. TADA, K. and AKAISHI, T. Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata 951

Our previous study (Brain Res., 262(1983):151-154) hsa comfirmed the existance of the recurrent inhibitory synaptic inputs in the about half of the paraventricular neurosecretory neurons. The present study was planed next to investigate the activity of the recurrent inhibitory system under different plasma osmolar state.

In 36.4 % of antidromically identified neurons (N = 11) which showed non-inhibitory phase following subthreshold antidromic stimuli, at 60 min after 0.6 M NaCl (1.0 ml, i.p.), clear inhibitory phase could be detected in the PSTH after subthreshold stimuli applied to the posterior pituitary gland. On the other hand, clear difference was not observed in the inhibitory phase of the remaining neurons (N = 4) which showed inhibitory phase at subthreshold stimuli before 0.6 M NaCl.

These data suggest that the activity of the recurrent synaptic inputs in the paraventricular neurosecretory neurons is varied under the different plasma osmolar state.

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INTERACTION BETWEEN SUCKLING STIMULI AND OSMOTIC STIMULI IN STIMULATING PARAVENTRICULAR NEUROSECRETORY CELLS IN RATS. NEGORO, H., HONDA, K., FUKUOKA, T.* AND HIGUCHI, T. Dept. of Physiol. Fukui Medical School, Matsuoka, Fukui 910-11.

Previously we have reported that osmotic stimuli may increase the amount of oxytocin released at each reflex milk-ejection but not the frequency of the recurring milk-ejections. In the present study the effect of 1.5 M NaCl (1 ml, i.p.) on the reflex activation of PVN oxytocin cells was studied in urethane-anesthetized lactating rats. Forty-seven antidromically identified units were recorded simultaneously with intramammary pressure during suckling before and after 1.5 M NaCl injection. Seventeen of them showed a characteristic burst of accelerated activity 12-20 sec before milk ejection and the magnitude of the burst response was increased following hypertonic saline injection. In ten of the units the reflex burst responses were observed without a detectable milk ejection; however, as the magnitude of the burst response was increased following 1.5 M NaCl, milk ejections began to occur in relation to the burst. The remaining units were identified as non-oxytocinergic cells. These results indicate that rise in plasma osmolality stimulates the reflex activation of oxytocin cells interacting with suckling stimuli.

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PROLACTIN AND OXYTOCIN RELEASE INDUCED BY SUCKLING STIMULI IN THE RAT. HIGUCHI, T., HONDA, K., FUKUOKA, T* AND NEGORO, H. Department of Physiology, Fukui Medical School, Matsuoka, Fukui 910-11.

The secretory profile of prolactin (PRL) and oxytocin (OXY) during nursing was studied in unanesthetized and urethane-anesthetized rats. Serum PRL levels were determined by radioimmunoassay. OXT release at the milk-ejection reflex was monitored by the intramammary pressure and/or characteristic pup's reaction associated with the reflex. Serum PRL began to elevate earlier than the first milk ejection in unanesthetized rats, but never increased without appearance of milk-ejection reflex in urethane-anesthetized rats. Pulsatile fluctuations of serum PRL levels with 6-15 min intervals were observed during the nursing period when 10 pups were continuously suckling. The intermittent milk-ejection reflexes occured not always but preponderantly when the serum PRL levels were at nadir of the fluctuations. Injection of estimated dose of OXT (1 mU) released at each milk ejection did not change the serum PRL levels. These results indicate that the release of both PRL and OXT is pulsatile in nature and that a common biological clock may be participated in the regulation of the pulsatile secretory rhythm.

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ADH-SECRETING CELL: EXCITATION AFTER NOXIOUS STIMULI IN RATS. HAMAMURA, M., SHIBUKI, K. and YAGI, K. Dept. of Physiol., Jichi Med. Sch., Minamikawachi-machi, Tochigi 329-04

Effects of noxious stimuli on discharge activity of electrophysiologically identified supraoptic ADH-secreting cells were studied in anaesthetized rats. Tail pinching (TP) evoked excitaion in 20 % of 39 phasically firing cells (P-cell) and about a half of 57 cells with slow and irregular firing pattern (SI-cell). All of the 7 SI-cells which temporarily showed phasic firing pattern excited after TP. TP also induced a decrease in arterial blood pressure (ABP) in some cases. However, the fall of ABP which is known to induce ADH secretion cannot explain all of the evoked excitation, since the excitation preceded the decrease in ABP. Peristimulus time histograms of unit discharges disclosed that sciatic, cutaneous and trigemial nerve stimulations produced excitation mediated by direct synaptic inputs in some P-cells. Since P-cells have been identified as ADH-secreting cells, it is suggested that ADH-secreting cells excite in response to noxious stimuli as the result of direct synaptic inputs originating from noxious afferents.

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CYCLIC AMP RESPONSE TO AVP AND PTH IN RAT RENAL CELL MONOLAYERS. HATANO, T., OGAWA, K., YAMAMOTO, M., KANDA, K., AND MATSUI, N. Res. Inst. Environ. Med., Nagoya Univ., Nagoya

The response of cAMP to arginine vasopressin (AVP) and $^{1-34}$human parathyroid hormone (PTH) was studied using rat renal cells in monolayer culture. Rat kidney cells were dispersed with collagenase at 37°C for 30 min. Cells in Dulbecco's Modified Eagle's Medium with 16% fetal calf serum were incubated at 37°C under 5% CO_2-air. Hormone stimulation was performed with 0.25mM 1-methyl 3-isobutyl xanthine. cAMP in cells after extraction with TCA and cAMP in media without any treatment were determined by radioimmunoassay. [Results] After 2 days of culture cAMP response to AVP was maximal but it declined thereafter. AVP and PTH stimulation resulted in a rapid increase of intracellular cAMP; a peak was found after 10 min with AVP and a plateau was reached after 5 min with PTH. Medium cAMP increased linearly by AVP and PTH. The response of cAMP to AVP was significantly higher in medulla than in cortex, while the response to PTH was remarkable only in cortex. An excellent sensitivity (10 pg/ml of AVP and 10 ng /ml of PTH) was obtained in this system. In medulla, LVP, DDAVP, and oxytocin at a dose of 10^{-9} M showed 36.1%, 154.2%, and 8.1% of AVP, respectively, which seemed corresponding to their antidiuretic activities. Eel calcitonin at the same dose also showed 149.1% of AVP in medulla. Present study demonstrated that this system provides a useful tool for investigating the mechanism of hormone-cell interactions in kidney.

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ROLE OF OSMOTIC PRESSURE GRADIENTS IN ACETYLCHOLINE RELEASE FROM FROG MOTOR NERVE TERMINALS AND CATECHOLAMINE SECRETION FROM BULLFROG ADRENALS. KITA, H., YASUGI, E. and NARITA, K. Dept. of Physiology, Kawasaki Medical School, Kurashiki, Okayama 701-01

When the bathing solution is abruptly changed from hypertonic to isotonic, the rate of spontaneous quantal acetylcholine release at the frog neuromuscular junction and catecholamine (CA) secretion at the isolated bullfrog adrenal are both increased transitorily. The transient increase in CA secretion in response to the osmotic pressure change has the following features. (1) It is dependent on the duration of soaking in hypertonic solutions. (2) Its magnitude is determined by the degree of hypertonicity. (3) It is also observed in Ca^{2+}-free, MgEGTA-containing solutions as well as in solutions whose sole divalent cation is Mg^{2+}. (4) Below 1 mM, it is independent of the concentration of Ca^{2+} in the bathing solution. (5) The transition from isotonic to hypotonic solutions produces the same transient increase. Hypertonic solutions inhibit high K^+-induced CA secretion while hypotonic solutions enhance it. These results are consistent with the observations in the liposome-bilayer experiments by Cohen et al. (1982) suggesting that an osmotic pressure gradient across the vesicle membrane could play a role in exocytosis which results in the release of neurotransmitters and hormones.

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LESIONS OF HYPOTHALAMIC NUCLEI MODIFIED GROWTH HORMONE (GH) RELEASE INDUCED BY STIMULATION OF VENTROMEDIAL HYPOTHALAMUS (VMH) IN RATS. KATO, M., SUZUKI, M. and KAKEGAWA, T. Dept. of Physiology, Inst. of Endocrinology, Gunma University, Maebashi 371

Stimulating electrodes were implanted in bilateral VMH and the jugular vein was cannulated for blood sampling one week prior to the experiment. At the same time lesions of the anterior periventricular nucleus (APN) or of the dorsal premamillary nucleus (DPN) were performed with an anodal current. The rats were pretreated with α-methyl-p-tyrosine to prevent spontaneous GH bursts. The stimulation of VMH for 10 min increased plasma GH concentration from 19 ng/ml to 275/ml by 10 min after the cessation of stimulation, while plasma GH level remained at the resting level for a short time after the termination of stimulus in normal rats. The apparent suppression of GH release during VMH stimulation was abolished and GH release occurred during the VMH stimulation in the APN lesioned rats. On the other hand, the VMH stimulation completely failed to raise plasma GH level in DPN lesioned rats, however the stimulation of DPN itself caused no change in the plasma GH level. These results indicate that the stimulation of VMH exerts an inhibitory effect on GH release through the APN and that the DPN is important in the GH release induced by the VMH stimulation.

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CNS MECHANISM IN RELATION TO GH AND TSH RELEASES STIMULATED BY CLONIDINE (II).
ISHIKAWA, K., KAKEGAWA, T. and SUZUKI, M. Dept. of Physiology, Inst. of Endocrinology, Gunma University, Maebashi 371

To further define the sites in the brain at which NE system acts to stimulate GH and TSH releases from the pituitary, the effects of electrolytic lesions in the various hypothalamic nuclei on the secretion of TSH and GH were tested in conscious unrestrained, α-MT treated rats. The injection of small dose (150 μg/kg, iv) of the α_2 agonist, clonidine, stimulated both TSH and GH releases. Placement of electrolytic lesions in the medial preoptic/anterior hypothalamus (m-PO/AH) where somatostatin neuronal perikarya is mainly located, significantly reduced the GH response to clonidine administration. In contrast to the GH release, the TSH response to clonidine was not inhibited by m-PO-AH lesions. The arcuate nucleus lesions also reduced significantly the GH response, but was ineffective for the TSH response. On the other hand, the lesions in the paraventricular nucleus abolished the TSH-releasing effect of clonidine, but not affected the GH response. These results suggest that the central noradrenergic system(s) is involved in two different sites for TSH and GH secretions.

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CONCERTED EFFECTS OF TRIIODOTHYRONINE(T_3) AND CORTICOSTERONE(B) ON THE DEVELOPMENT OF NEWBORN RAT BRAINS.
TAKAHASHI, T., GOTO, K., SUDO, S. and NAKANO, S. 2nd Dept. of Physiol., Kanazawa Med. Univ., Uchinada-cho, Ishikawa 920-02

Corticosterone(B) and T_3 have profound effects on the development of brains during neonatal life, but the effects of the two hormones are different.

The administration of B(3 μg/g body wt/day) between 0 and 9 days old raised protein, DNA and tubulin contents(per wet wt) in soluble fractions from 10 day-old cerebellum and also protein(per wet wt and per DNA) in the cerebrum. The T_3(50 ng/g body wt/day)-treatment raised not only those parameters but also RNA(per wet wt) in cerebellum and tubulin(per wet wt and per DNA) in hypothalamus from 10 day-old rats. If the two hormones were administered together during the same period, the treatment raised protein (per wet wt) in the three areas, tubulin(per wet wt) in the cerebrum and cerebellum and tubulin(per DNA) in the cerebellum, but did not change DNA contents in 10 day-old rat brains. When B or T_3 were administered between 10 and 19 days old, a little effects were observed in 20 day-old rat brains, but if B and T_3 were administered together during the pariod, no parameters were changed in the 20 day-old brains.

Although the two hormones have different manners of actions each other, the B + T_3 treatment increased protein and tubulin contents(per DNA) but not DNA in 10 day-old rat cerebellum. However, normalizing effects on the parameters were observed in 20 days old.

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STUDIES ON THE ROLE OF VISION FOR THE POSTURE. KOSEKI, K., SAKAMOTO, M.* and ITOW, H. Body Reformatory School, Meguro-ku, Tokyo 153

At the opened-eyes a correspondence exists between the distribution of foot-pressure and the upright posture, and the upright posture can be classified in six types. We tried to examine whether the same result might be got also at the closed-eyes.

The foot-pressure was measured by the microcomputor using the ultra-microtransducer of load.

The measuring part covers 20 points on each sole, and we recorded at every second during thirty seconds.

We classified the upright posture by the balance method.

The subjects are mostly the teachers and clerks of our school.

Now we found the following facts.

The difference of change of foot-pressure at the closed-eyes is larger than that at the opened-eyes.

The balance of before and behind is better at the closed-eyes than at the opened-eyes.

We think also at the closed-eyes a correspondence exists between the distribution of foot-pressure and the upright posture, though not so distinctly as at the opened-eyes.

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DEPENDENCE ON CENTRAL CONTROL OF AUTOREGRESSIVE ACTIVITY OF STEP LENGTH IN TREADMILL WALKING. YAMASAKI, M., SASAKI, T., TSUZUKI, S. and TORII, M. Dept. of Physiol., Inst. of Constitutional Med., Kumamoto Univ., Kumamoto 862

A thousand step lengths in treadmill walking were successively measured and the data in time series were analyzed by application of autoregressive method to reveal the relationship between central activities and rhythmic pattern of walking. The measurement system of step lengths consisted of a position sensitive device, shoes with a foot switch and a minicomputer. Six adult subjects were tested at fixed speed levels, ranging from 60 to 120m/min with an increment of 10m/min or 20m/min. Autoregressive power spectrum of step lengths and its component waves during free walking revealed a dominant peak at a periodicity of two steps, and the residual power in other periods was small enough to be ignored. In order to demonstrate association of central control, steps were counted silently during walking by repeating a set of counting from 3 to 7. Power spectrum generally revealed a peak at a periodicity of respective number of steps in the set as well as at two steps. The former peak was dominant on walking at a cycle of 3 or 4 steps, diminished progressively and finally disappeared at 6 or 7. These observations definitely indicate that mental activity coordinates and controls the organic systems involved in walking.

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THE RELIABILITY OF THE STATIC MUSCULAR ENDURANCE TEST AS A FUNCTION OF MEASURING THE WORK LOAD OF EXTENDING ARMS (HANGING DOWN). USUI, S., HARADA, K., TOKIOKA, A.* Lab. of Phys. Ed., The Jikei Univ. Sch. of Med. Kokuryo-cho, Chofu-shi, Tokyo 182, SAKAI, T., Dept. of Physiol. The Jikei Univ. Sch. of Med. Nishishinbashi, Minato-ku, Tokyo 105.

For the purpose of studying the reliability of the static muscular endurance as a function of measuring the work load of extending arms (hanging down), first of all we studied the accuracy of the measurement values by the same subject under the regulation environment. As a result, the body weight (BW)(70.68±0.86kgw) and the grip strength (GS) (59.89±1.05 kgf) increased in a small way in the latter term of measurement, but the maintenance time of extending arms (111.57±5.05Sec) changed little. Therefore, neither GS/BW value (0.848±0.015) nor the work load of extending arms value (7883.37±304.55kgw·Sec) and the work load of extending arms/Gs value (131.68±5.50kgw·Sec/kgf) changed greatly. From these results, we concluded that the work load of extending arms value has a high reliability as the parameter of the static muscular endurance and we applied the parameter to the adult males (102 persons).

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EVALUATION ABOUT MECHANISM OF RENAL URINE CONCENTRATION AND DILUTION AFTER ANAEROBIC EXERCISE. INOMOTO,T. and OGO,K. Dept. Morphol.,Inst. Const., Med., Kumamoto Univ., Kumamoto 862, Dept. Health and Physical Educ., Fac. Educ. Kumamoto Univ., Kumamoto 860

The relation between the renal urine concentration and dilution and the anaerobic power was investigated in six male sprinters, aged 18-26 years. The anaerobic power(oxygen debt)was measured following two different exercise. One was long time exercise(15-18min) on a bicycle ergometer, another was 300meters full speed running(35-40sec), both up to 30minutes after exercise. The fall of GFR was observed in exercise on a bicycle ergometer, and post/pre-ratio of urinary volume (30min after exercise/rest)ranged from 0.18 to 1.07. Then the fall of GFR was observed in 300meter sprint,but urinary volume increased after exercise, and post/pre-ratio ranged from 1.16 to 5.75. The correlation coefficients between oxygen debt and urinary volume ratio were ranged from $r=0.814$ to $r=0.994$ ($p<0.001$),respectively. This may be a result from the fall on renal tublar reabsorption of water.

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THE RELATION OF EXCESS CO_2 TO BLOOD LACTATE AND O_2 DEBT. NARUSAWA,M.,TERAO,T.,NAGAMI,K. and NAKANO,S. Dept. of Physiol., Tokai Univ. Sch. of Med., Isehara 259-11, Kanagawa.

It's well known that the higher RQ was shown with the increasing of O_2 uptake and the RQ over than 1.0 was measured during heavy exercise frequently. The accumulation of lactic acid with lowerring of bicarbonate pool in body may be caused by the higer increase of CO_2 output than the O_2 uptake. As if the metabolic RQ is assumed with in 1.0, the amount of excess "nonmetabolic" CO_2 will be measured over RQ 1.0 from that CO_2 product increased. This study was designed to clarify the relation of excess CO_2 to the blood lactate at the submaximal work. After detamination of Vo_2 max with the leg exercise on a bicycle ergometer, three different degree loads(60, 80 and 95% of Vo_2 max) were examined respectively. All subjects studied for 5 min at the assigned work load. Blood sumples were taken from v. mediana cubiti at six times of each trial. The concentrations of blood lactate and glucose were determined by the Somogyi-Nelson and the Barker-Summerson methods. The results were as follows: (1) The increase of lactate was correlated with the rise of excess CO_2. (2) The neutralized lactate calculated with the excess CO_2 accounted for about 25% of total body lactate. These results suggested that the excess CO_2 that was generated by the entry of hydrogen ions into the body fluids will stoichiometrically to product of the lactic acid.

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CHANGES IN WATER AND ELECTROLYTE METABOLISM AND RELATED HORMONES TO A REPEATED EXPOSURE TO ALTITUDE. TAMURA, Y., OKAZAKI, S., HATANO, T., KANDA, K., OGAWA, K., SEO, H. and MATSUI, N. 2nd Dep., Res. Inst. Environ. Med., Nagoya Univ., Chikusa-ku, Nagoya 464

To investigate the mechanism of adaptation, 5 male climbers were exposed to 6,000m simulated altitude (2hr-ascent, 2hr-stay, 2hr-return) in 3 successive days. 4 blood and 3 urine samples were subjected to assess water and electrolyte metabolism and hormone responses. 1) Responses to 1st exposure: Blood Hb, protein, osmolality immediately rose following the exposure indicating a plasma volume decrease. Rises in serum Na and Cl and a fall in serum K may have been caused by hyperventilation induced alkalosis. Serum aldosterone, cortisol and adrenaline rose markedly whereas the rise in PRA was small and change in noradrenaline was inconsistent. 2) Responses to 2nd and 3rd exposures: Responses of Hb, protein and osmolality were larger than the 1st, however, changes in basal levels (progressive decrease in Hb and protein and progressive increase in osmolality) made the comparison difficult. Responses in electrolyte slightly decreased but again this statement should be reserved by the same reason. On the other hand, responses in cortisol, aldosterone and adrenaline progressively diminished. From the results it may be concluded that stress-induced hormone secretion decreases by repetition of stimulation, however, changes related to other factors are difficult to evaluate because of alterations in fluid metabolism (hyperosmolar overhydration) by previous stimulation.

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REDUCTION IN MAXIMUM HEART RATE AND WORK CAPACITY UNDER ACUTE EXPOSURE TO HIGH-ALTITUDE ENVIRONMENT.
MORI, S., TAKABAYASHI, A., MITARAI, G. and *SAKURAI, S.
Dept. of Aerospace Physiol., Res. Inst. of Environ. Med., Nagoya Univ. and *Res. Center of Health, Physical Fitness and Sports, Nagoya Univ., Chikusa-ku, Nagoya 464.

Heart rate increase in response to moderate exercise (300, 450 and 600 kpm/min, for each 3 minutes successively) was facilitated with increasing simulated altitudes in a decompression chamber, until it reached a ceiling of around 160 beats/min above 6,000 m. The ceiling value was remarkably lower than the maximum heart rate (HRmax) determined at sea level (194 beats/min averaged for 12 subjects). The reduction rate was close to that shown previously for chronic hypoxia. A reduction in work capacity was also estimated for such suppression of the heart rate (1,080 kpm/min at 6,000 m against 1,530 kpm/min at sea level). After returning from a high-altitude expedition (4 days after descent, 6 subjects), the ceiling for the heart rate increase was similarly observed during the acute exposure to simulated altitudes and the HRmax at sea level remained unchanged, despite of significant improvement in the work capacity, suggesting that mechanisms for the limitation in heart rate increase under hypoxia might be independent of acclimatization.

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The contractile properties and the discharge pattern of single motor units in humans.
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MASUDA,M. Dept. of Physiol., Jikei Univ. Sch. Med., Minato-ku, Tokyo 105

The contractile properties and the discharge pattern of human motor units(MUs) from m. abductor digiti minimi(ADM), m.extensor digitorum(ED) and m.deltoideus(D) of upper arm were studied during voluntary isometric contractions. The coil-chaped inserted electrode was used for the record of spike potentials of MUs. The contraction time(C.T.) and the peak tension(P.T.) of the indivisual MUs were determined from the twitch tension curve obtained by averaging the sustained force. Threshold force(Fth) of MUs were ditermined by the force at the moment of the recruitment of each MU during ramp force exertions in each gradient. The gradient of the tension increase were at 100g/s in ADM and ED, at 1kg /s in D. There was no significant correlation between C.T. and Fth, a wide range of C.T. was observed in each muscle. The relationship between Fth and P.T. tended to be nearly linear in ADM and D, but the range in ED was fixed within 20g. The wide range of C.T. in ADM, of P.T. in ED and of C.T. and P.T. in D were observed. The deviation in interspike interval was small in MU with a low Fth, but large in MUs with a high Fth in D. The large deviation in interspike interval was observed in ADM regardless of MU with a low Fth. MU with a low The was observed in large deviation of interspike interval and small deviation in MU with a high Fth in ED. It was suggested that relationships between the contractile properties and the discharge pattern of each MU were different in each muscle.

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VOLUNTARY SINGLE HUMAN MOTOR UNITS
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MASUDA, M. and SHIBAYAMA, H., Dept. of Physiol., Jikei Univ. Sch. Med., Minato-ku, Tokyo 105

To examine the changes in the recruitment threshold of single human motor units produced by various cutaneous stimulations, single human motor units of the first dorsal interosseus muscle were examined during voluntary contractions using coil-shaped inserted electrodes. Isometric abduction forces of the index finger at the proximal interphalangeal joint during sustained and ramp forces at 100 g/s detected by a force transducer were recorded simultaneously with action potentials of motor units.

Twitch tension curves of single motor units in the first dorsal interosseus muscle were obtained by the spike trigger averaging method. Motor units examined in this study had the threshold forces of ranging from 2 to 1000 g, contraction times ranging from 50 to 80 ms and peak tensions ranging from 0.3 to 6.1 g.

Changes in the threshold force of single motor units in the first dorsal interosseus muscle produced by cutaneous electrical, pressure, cold and pain stimulations were observed.

It was suggested that cutaneous pressure, cold and pain sensations might play their own roles to change in the threshold force of motor units in the organization of synaptic input to the α motoneuron pool.

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RELATION BETWEEN THE FORCE VELOCITY AND THE DISCHARGE RATE OF EMG DURING VOLUNTARY ISOMETRIC CONTRACTION IN MAN. T.YONEDA, M.KIMURA, K.OISHI AND A.ISHIDA. Dept.of Physiol., Sch.of Physical Edu. Juntendo Univ. Fujisaki, Narashino, Chiba.

The force velocity-e.m.g. relation during voluntary isometric contraction of Tibialis Anterior was studied. The subjects employed in this experiments were four healthy male adults. The force levels were set with a range of 10-55% of MVC. The subjects controlled the force and speed of exertion by referring to an oscilloscope that was monitoring their force exertions. The forces performed within 150 msec time to peak were defined to ballistic contraction and the other forces over 151 msec time to peak were defined to the ramp contractions.

In ballistic contractions, the amounts of discharge(i.e.m.g.) varied, while those increased with the time to peak increment in ramp contractions. The discharge rates(mv/msec) varied when ballistic contractions, although those were nearly constant when ramp contractions. These results were similar to the our previous report that had been derived from the hand muscle experiment. It is suggested that the muscle discharge properties during ballistic contraction differ from those during ramp contractions.

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EFFECTS OF MAXIMUM VOLUNTARY CONTRACTION ON EVOKED EMG AND TORQUE DURING THE PLANTAR FLEXION. NAGATA, A. and MURO, M. Bio-dynamics Lab., Faculty of Science, Tokyo Metropolitan University, Yagumo, Meguro-Ku, Tokyo 152

2 min. work of Maximum Voluntary Contraction(MVC) with the plantar flexion were practiced in the middle time of the electric stimulus, and then some estimating coeficients about muscular contraction were investigated quantitavely before and after this work. Acute short-termed fatigue was happened by this MVC work and was identified with frequency shift in the mean power spectrum of the fatigued EMG of the soleus. Before and after this fatigue, the electric stimulation was added to the tibial nerve at the knee joint and at the same time, the evoked EMG(H and M waves)and generated torque with the plantar flexion were recorded from the soleus on the memory scope and x-y plotter.

From analyzing the fatigued EMG spectra, F and S types of the power spectra pattern were classified with frequency shift to be appeared muscle fiber types of the soleus, and then according to these types, many physiological parameters were measured comparatively. H/M ratio, Torque/M, Torque/H, Electro-Mechanical Delay(EMD, M-wave, H-wave and Voluntary Reaction) and Rise Time(RT, M, H and VR) had been effected severely by the measure of the heavy work load. Especially, T/M valeus have been shown increasingly and EMD has been decreased with muscle contraction velocity after this work. Neuro-muscular function would be changed significantly at the fatigued physiological condition.

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RELATIONSHIP OF THE FATIGUED MUSCLE BETWEEN THE STRETCHING LEVEL AND THE EVOKED EMG CHARACTERISTICS. MURO, M. and NAGATA, A. Bio-dynamics Lab., Tokyo College of Pharmacy, Hachioji-city, Tokyo 192-03

Relationship between muscular stretching level and characteristing of electrically evoked EMG (H- and M-wave), have been studied about triceps surae of 10 healty young subject. H- and M-wave amplitude were measured comparatively before and after muscular fatigue. The nerve stimulation were used with rectangular voltage pulses (0.5msec duration). For each subject the nerve stimulus intensity was adjusted to be maximal threshold of the evoked H- and M-amplitude in the soleus and the gastrocnemius.

The experimental results indicated that H-reflex amplitude was dependent on the amount of the muscle stretch and stretching duration, and then H- and M-wave amplitude were effected to be decresed and increased with the stretching respectively. These phenomena could be explained to be the inhibitory of the neuro-muscular function from the peripheral sensory nervous system. With the muscular stretching, nervous depression in the excitability (motor-neuron of soleus and gastrocnemius) and length change in the sarcomere should be happened in the motor neurons of the spinal cord and in the muscular fibers on the same time.

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Circadain rhythm of running wheel activity in Spontaneously-Hypertensive-rats and Wistar Kyoto-rats. ISOBE, Y.* , OHARA, K.*, HASEGAWA, Y.†, and AOKI, K.§ 2nd* and 1st† Dept. of Physiol. and Dept. of Medicine § , Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya, 467

Circadian rhythm of running wheel activity during the light(L)-and-dark(D) cycle and continuous light(LL) conditions showed different manner in Spontaneously-Hypertensive-rat (SHR) and Wistar-Kyoto-rats (WKY); i.e. an entrainability to LD cycle was higher in SHR than that in WKY, and the circadian period (τ) under LL was longer in SHR (25.20 h) than that in WKY (25.05 h), the active period (α) was shorter in WKY than in SHR. Relation of circadian period and active period was significantly different in SHR and WKY. Separately, freerunning rhythm was measured under the ambient temperature of 16, 25, and 30 °C. Splitting phenomena were observed under 30 °C in one out of eight SHR, and five out of seven WKY after the 10 days of LL was begun. Freerunning period under 16 °C showed longer period than that under the 30 °C in both strains. In the SHR, negative correlation coefficient was found between ambient temperature and circadian period. Ratio of the circadian period against the changes of ambient temperature of 10 °C was remarkably close to 1.00 in both strains. These results indicate that the nature of the oscillator is different in SHR and WKY, and suggest that the oscillator is well temperature compensated.

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THE EFFECT OF EXERCISE ON PULSE WAVE VELOCITY IN SPONTANEOUSLY HYPERTENSIVE RATS(SHR). Fujita,Y., Jikihara,K., Ishihara,A., Takemiya,T. & Fujita,T. Div. of Physiol. Inst. of Health & Sports Sci. The Univ. of Tsukuba. Ibaraki. 305.

The rats were devided into three groups at 6 weeks of age; SHR exercise(Ex), SHR non-exercise(nE) and Wistar control rats(WCR), which were devided again according to sex, respectively(Total 6 groups). SHR-Ex were placed in cages with rotating wheel as they were able to exercise voluntarily. All groups were raised untill 30 weeks. Pulse Wave Velocity(PWV) were measured upon aorta in each diastolic blood pressure which was varied making use of Clonidine injection and bleeding. Following the experiment, histological evaluations were performed on their thoracic aorta using cross section preparation. PWV of SHR-nE(6.73m/s at 100mmHg) was significantly higher than that of SHR-Ex(5.86m/s) and WCR(5.40m/s) in male. Thickness/diameter ratio of thoracic aorta of SHR-nE(male 7.64%) was also significantly greater than that of SHR-Ex(6.59%) and WCR(6.06%). Elastic fiber rate of female SHR-nE(23.4%), which was measured on section of Elastica Van Gieson, was greater than that of male SHR-nE(19.9%). No degeration was discernible upon intima, observed by Sudan III preparation.

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HIGH ALTITUDE ADAPTATION OF SHR. MOTOYAMA,T., SAKAI,A., YANAGIDAIRA,Y., YONEKAWA,M., UEDA,G., KOBAYASHI,T.*, KUBO,K.*, YOSHIMURA,K.* and YASAKI,K.* Dept.of Adapt. Physiology, Dept.of 1st Medicine*, Shinshu Univ. Sch. Medicine, Asahi, Matsumoto, 390

Wistar (WR) and spontaneously hypertensive rats (SHR) of 4 weeks old (n=96) were raised at a control level (610 m) for 90 days and/or Yatsugatake (2,400 m) for the latter 60 days. During the stay at altitude the decrease of the body weight was greater in SHR than in WR. The systolic blood pressure, measured at the tail artery, of WR decreased from 103 mmHg at control to 92 mmHg at altitude, namely the decrease was about 11 %. In the mean time, that of SHR decreased from 174 mmHg at control to 123 mmHg at altitude, namely the decrease was about 29 %. In SHR, the left ventricular hypertrophy is relatively greater than in WR. Thus the ratio, total ventricular weight / body weight, indicated greater values in SHR than in WR. At altitude, the left ventricular weight remained unchanged, but the right ventricular weights increased in both groups especially in WR. Therefore, high-altitude raised rats revealed higher values of the ventricular weight than those of the control. From these, morphological and functional adaptations at altitude were ascertained in both cases.

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PROTEIN METABOLISM ON PAPUA NEW GUINEA HIGHLANDERS. MIYOSHI,H. OKUDA,T. KAJIWARA,N. MIYATANI,S. and KOISHI,H. Fac.of the Sci. of Living, Osaka City University, Sumiyoshi-ku, Osaka 558

Papua New Guinea Highlanders(PNG) have healthy, well-developed physique. Their staple food is sweet potato and their protein intake is about 0.6g/kg/day. It was studied to make clear the mechanism for adaptation to such food habit. The experiments were designed to feed some levels of protein and two kinds of diet composition to Japanese (JPN) and PNG. Nitrogen balance, blood status, incorporation of ^{15}N into serum protein and the excretion of ^{15}N in urine and feces were measured after ^{15}N-labelled urea administration orally.

The urinary and fecal excretion of ^{15}N was greater in JPN than in PNG and was greater on adequate protein intake(1.3g/kg) than on low protein intake(0.5g/kg). There was no difference between JPN and PNG in ^{15}N incorporation into serum protein on low protein intake. On adequate protein intake, ^{15}N uptake in serum protein of PNG was similar to on low protein intake but, in contrast to PNG, ^{15}N uptake in serum protein was not detected on JPN. When PNG were fed adequate protein diet for 13 days, the nitrogen accumulation was remarkable and the concentration of free essential amino acid in serum was kept low level.

These data suggested that PNG have high ability of nitrogen accumulation than JPN.

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Effect of diet composition administered before exercise on prolonged running. OJIRI, Y., YAMASHIRO, M.*, SHINJO, S.*, SAKURAI, T.*, and SUGIURA, M. Dept. of Health Physiol. and Nutrition, Ryukyu Univ. Med. Sch., Nishihara-cho,Okinawa 903-01

High carbohydrate diet was administered to six volunteeres for 3 days before the tests to increase the amount of muscle glycogen. Then, they were subjected to exercises at 65% (test I) and 80% (test II) of maximum oxygen uptake after pre-exercise high carbohydrate diet (pre-HCD) and after pre-exercise high protein diet (pre-HPD). Metabolic and respiratory parameters were measured during exercise. Performance time after pre-HCD was longer than that after pre-HPD in the test II. The respiratory exchange ratio after pre-HPD was lower than that after pre-HCD in both tests. In the test II, glucose and NEFA after pre-HPD were higher than that after pre-HCD. On the contrary, lactate was lower in pre-HPD. The results indicate that food constituents, particularly the constituents of diet taken just before exercise, have an important role in the efficiency and performance of prolonged exercise.

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VOLUNTARY AMINO ACID INTAKE IN RATS FED DIETS DEVOID OF THAT AMINO ACID. KISHI, K. and INOUE, G. Department of Nutrition, School of Medicine, The University of Tokushima, Kuramoto-cho, Tokushima 770

The regulation of amino acid intake was studied in rats given diets devoid of one essential amino acid. Growing rats of Wistar strain, weighing about 80g, were given a diet deficient in one of ten essential amino acids together with drinking water and an amino acid solution deficient in the diet for 14 days. Control rats were fed a complete amino acid mixture diet simulating egg protein with drinking water. Total amino acid levels of basal diet were 3, 7, 10 and 15% as N x 6.25. The rats consumed amino acid solution more than drinking water. Food intakes of lysine, arginine and histidine groups were comparable with control, but those of isoleucine, phenylalanine and leucine groups were lower. Voluntary amino acid intake from the solution was paralleled the total amino acid level of the diet in all essential amino acid tested. Each amino acid intake expressed as A/E ratio (an essential amino acid/total essential amino acids) was similar to the amino acid requirement pattern published by NRC (1978) except that lysine intake was 150% and phenylalanine intake was 75% of NRC pattern. Similar growth curve was shown in rats given amino acid mixture diet obtained in this study and egg pattern amino acid mixture diet.

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CARDIOVASCULAR TISSUE FIBERS IN OLD EXERCISED RATS. FURUI, H., SATŌ, T., HASHIMOTO, M., TAKAHASHI, K. AND MASUMURA, S. Dept. of Physiology, Shimane Medical University, Izumo 693

In old female Wistar rats(86 weeks old), fibrous proteins(collagen and elastin) in the aorta and ventricles were estimated after forcing the rats to run on a treadmill. In the initial step of running exercise, rats ran at 7-20 m/min with a slope of 10° for 1 hr/day, 6 days/week. After 4 weeks, the same rats were made to run continuously at 20 m/min for 1 hr/day. The entire experiment lasted 12 weeks. The estimated values of collagen and elastin were expressed in terms of percentage of dry defatted weight.

With respect to sedentary rats, collagen content was 29.9 % in the thoracic aorta, 43.8 % in the abdominal aorta and 3.6 % in the ventricles, while elastin content amounted to 33.5 % in the thoracic aorta and 25.6 % in the abdominal aorta. In this case, elastin content in the ventricles was hardly detected. The control values of elastin in the aorta decreased by 30-40 %($p<0.05$) in comparision with those found in young rats(5 weeks old)(Masumura et al. Experientia(1983)). After forced running the estimated values of these fibers were unchanged in the aorta while the amounts of collagen in the ventricles decreased by 14-15 % of the controls($p<0.05$). This finding indicates that running exercise is effective in reducing collagen in the ventricles.

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CHANGES IN FATTY ACID COMPOSITION OF MYOCARDIAL PHOSPHOLIPIDS OF TRAINED RATS. IWAGAKI,S.and OGAWA,K.Dept.of Physical Recreation and Health,Faculty of Physical Education,Tokai University, Hiratsukashi,Kitakaname 1117.

Wister strain male rats were swum every day for 7 weeks. By control of the swimming times, the rats were divided into two groups, group A and B. Group A showed similar body weight to sedentary rats, but that of group B lowered significantly ($p < 0.05$). Effects of the training on fatty acid composition of myocardial phospholipids were studied. Fatty acid compositi -on of myocardial phospholipids on group A showed significant increase of linoleic acid composition of cardiolipine(CL), phosphatidylethanolamine(PE), and phosphatidylcholine(PC). On the group B, there were not changes in fatty acid composition of PE and PC, but on that of CL, linoleic acid composition decreased reversely. By refrigerated centrifugation, 5000 and 30000×g fractions of these muscles showed similar composition to that of PE. As these results, training caused changes in fatty acid composition of cellular component lipids, and especially main effects appeared on linoleic acid composition of the cellular component lipids. These changes suggest that functional changes by training are related to changes in fatty acid composition of cellular component lipids, phospholipids.

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EFFECT OF PHYSICAL TRAINING ON LIPOLYSIS INDUCED BY VARIOUS LIPOLYTIC AGENTS. IZAWA, T., KOSHIMIZU, E., *KOMABAYASHI, T. and *TSUBOI, M. Dept. of Physical Educa. and *Dept. of Pharmacol., Tokyo Coll. of Pharm. Hachioji, Tokyo 192-03

Lipolysis induced by various lipolytic agents in epididymal adipose tissue(WAT) of physical trained rats and chronic isoproterenol-treated rats was investigated. Physical training(running 5 times/wk for 8 wk) reduced weight-gain and amount of WAT and interscapular brown adipose tissue(BAT) and caused hypertrophy of heart, whereas chronic isoprote-renol-treatment(2.5mg/100g body wt/day sc for 6 days) reduced amount of WAT and BAT and caused hypertrophy of heart and submandibular gland. Physical training markedly increased lipolysis induced by adrenaline and slightly increased lipolysis induced by noradrenaline and caffeine. But lipolysis induced by ACTH was markedly decreased. Isoproterenol-treatment markedly increased lipolysis induced by each lipolytic agent.

These results suggest that β-effect of cathecolamines is probably not the only mediator of response of rat epididymal adipose tissue to physical training.

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CHANGE OF LIPOPROTEIN METABOLISM IN SUBMAXIMAL EXERCISE. TERAO,T.,NARUSAWA,M.,NAKAHARA,Y. and NAKANO,S. Dept. of Physiol., Tokai Univ. Sch. of Med., Isehara 259-11 Kanagawa.

Recently, many studies were reported in the relationship of energy expenditude and lipoprotein(cholesterol, Cho.) metabolism; that were HDL-Cho. in serum increased in well-traineds. The HDL-Cho. was remarked as the negative risk factor to the coronary heart disease. This study was designed to clarify the relation of the exercise to the composition of lipoprotein; (1)The changes of Cho., triglyceride(TG) and phospholipid (PL) in lipoprotein on the non-athletes and long-distance runners groups at rest. (2)The variation of composition of the lipids(Cho., TG and PL) in each lipoprotein fraction (VLDL, LDL and HDL) during a assigned load(60% of $\dot{V}O_2$max, 30 min on the bicycle ergometer). The results were as follows; (1)In the long- distance runners group, Cho., TG and PL in VLDL were lower($P<0.01$) and Cho. and PL in HDL were higher($P<0.05$) than the non-athletes group at rest. (2)The relation of TG in VLDL to Cho. and PL in HDL showed negative correlation apparently. (3)After exercise, TG in VLDL on the long-distance runners group decreased than the control(rest) value. These results were suggested that the higher Cho. in HDL on the long-distance runners group might be caused to accelerate the transfer activity of a part of PL and Cho. from VLDL during the catabolism of TG in VLDL with lipoprotein lipase activity.

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GLYCOLYTIC ACTIVITY IN THE INITIAL PHASE OF AORTIC CONTRACTION. HASHIMOTO, M. Dept. of Physiology, Shimane Medical University, Izumo 693

Spiral strips of rabbit thoracic aorta were contracted by adrenaline(5×10^{-6}M) under aerobic conditions. This contraction developed a half of the maximum at 15-20 sec and reached the maximum at 10 min after the addition of this catecholamine. Within 2 min, the rate of lactate production in the contracting aorta increased by 280 % of the controls. On the other hand, the activity of PFK(one of the rate-limiting enzymes) increased by 30-50 %, while the level of Glc-1,6-P_2 also augmented by 30-50 % in the case of the experiment. The maximum level of Glc-6-P(200 % of the controls) was observed in the first 2-3 min and reduced to the control level after 10 min. The ATP content decreased by 30 % at the first 30 sec, whereas it was unchanged after 3 min in comparison with the controls.

In the initial phase of the contraction, these findings suggest that the augmented levels of these metabolites (Glc-6-P, Glc-1,6-P_2) depend on the stimulation of glycogenolysis and also suggest that the activation of PFK is related to the increased level of Glc-1,6-P_2(one of the strongest activators of PFK). In the initial phase of the contraction, the rate of ATP hydrolysis appears to be remarkable. Thus, it should be emphasized that the metabolic process in the thoracic aorta would be progressively proceeded immediately after the contraction.

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HUMAN TYMPANIC MEMBRANE TEMPERATURE IN COLD ADAPTATION. UCHINO, K. Faculty of Student Health Center, Yokohama National University, Hodogayaku, Yokohama 240. MASUDA, M. and HASEGAWA, H. Dep. of Physiology, The Jikei University School of Medicine, Minatoku, Tokyo 105.

The tympanic membrane temperature(Tty) had a increase 1 °C of ambient temperature from 23 °C to 36 °C. The changes of temperatures on the head and limb was unlike Tty. Tty was more varied than rectal temperature during warming or cooling. The decrease in Tty and shivering were occured at head cooling by a ice bag pillow, face immersion in cold water and ice bag forehead cooling during the bicycle exercise in normal subjects, but there was no change in Tty and no shivering in trained swimmers. The trained swimmer would be acquired the cold adaptation. It is considered that cold venous blood of the facial and head regions flowed to cavernosus sinus and then it cooled the blood of internal carotid artery, and consequently the hypothalamic temperature and Tty were decreased. But cold facial skin venous blood will be not flow to cavernosus sinus by heat insulation of deep skin vein in cold adapted swimmers.

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NOREPINEPHRINE-INDUCED THERMOGENESIS IN BROWN ADIPOSE TISSUE SUPPRESSED BY FASTING IN RATS. HAYASHI, M., JAVADY, M. AND NAGASAKA, T. Dept. of Physiol., Kanazawa Univ., Kanazawa 920

Fasting-induced changes in thermogenic responses to norepinephrine (NE 4 μg/kg·min, iv) were studied in anesthetized previously cold-acclimated rats. The rats were divided into 5 groups at the end of 30-40 days of cold-acclimation (5°C). The 5 groups were maintained for 5 days at 25°C and fed, or fasted, or fasted with daily treatment with thyroxine (T_4 2 μg/kg, sc), or thyroidectomized and fed, or thyroidectomized and fasted. In the intact fasted group, NE-induced increases in oxygen consumption, colonic temperature and temperature of the interscapular brown adipose tissue were markedly suppressed. Two thyroidectomized groups also showed low thermogenic responses. In the T_4-treated fasted group, fasting-induced suppression of thermogenic response to NE was partially prevented. Plasma levels of thyroid hormones were significantly lower in the intact fasted group than in the intact fed group. In the T_4-treated fasted group, although thyroxine was higher than in the intact fed group, triiodothyronine was lower. These results suggest that fasting-induced suppression of thermogenic response to NE is largely due to the reduced calorigenic response of the brown adipose tissue to NE. Fasting-induced lowering of levels of the thyroid hormones may be one of a number of causes of the reduced calorigenesis of the brown adipose tissue.

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ROLE OF SYMPATHETIC NERVOUS SYSTEM IN IMMOBILIZATION- AND COLD-INDUCED BROWN ADIPOSE TISSUE THERMOGENESIS OF RATS. SHIBATA, H. and NAGASAKA, T. Dept. of Physiol., School of Med., Kanazawa Univ., Kanazawa 920

The effects of chemical sympathectomy with 6-OHDA (100 mg/kg, ip) on 10-min cold (5°C)- or 2-min immobilization-induced thermogenesis in the brown adipose tissue (BAT) were studied in warm (25°C)-acclimated rats. Both cold- and immobilization-stresses increased heat production (M), interscapular brown adipose tissue temperature (T_{bat}) and colonic temperature (T_{col}). By both stresses, the increase in T_{bat} was greater than that in T_{col}, the difference (ΔT_{bat}) was approximately 0.5°C, which indicated thermogenesis in the BAT. In the sympathectomized rats, the 2-min immobilization did not increase ΔT_{bat}, but the 10-min cold exposure increased ΔT_{bat} by approximately 0.3 °C. This rise in ΔT_{bat} following cold exposure in the sympathectomized rats was not suppressed by a bilateral adrenalectomy with a supply of cortisone acetate (25 mg/kg·day, sc). These results indicate that the mechanism for BAT thermogenesis following immobilization may be different from that after cold exposure. Immobilization-induced BAT thermogenesis may be mainly controlled by the sympathetic nervous system to the BAT. However, cold-induced BAT thermogenesis seemed to be controlled partially by other mechanisms, besides the sympathetic nervous system.

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MECHANISM OF ADRENOCORTICOID-INDUCED THERMOGENESIS IN RATS. OHNO, T. and KUROSHIMA, A. Division of Nutrition Physiology, Hokkaido Educational College, Hokumoncho 9, Asahikawa 070 and Dept. of Physiology, Asahikawa Medical College, Nishikagura, Asahikawa 078-11

Metyrapone (11β-hydroxylase inhibitor) administration caused significant increase in $\dot{V}o_2$ of cold-acclimated rat (CA), but did not in warm control (WC) and heat-acclimated rat (HA). This result implies a role of adrenocorticoids, especially deoxycorticoids , in the cold-induced nonshivering thermogenesis (NST). Metyrapone increased blood glucose levels in all groups, but the extent of increment was less in CA and HA than in WC. Less increase in blood glucose of CA was related to the increase in resporatory exchange ratio, suggesting an enhanced glucose utilization in metyrapone-induced thermogenesis in CA. Furthermore, decrease in FFA (free fatty acid)/Glycerol molar ratio in metyrapone -treated CA suggested that an increased utilization of lipid is responsible for the metyrapone-induced thermogenesis.

From these results it is inferred that some adrenocorticoids are involved in cold -induced NST through regulating glucose and FFA metabolism. Single injection of deoxycorticosterone or corticosterone could not simulate the effect of metyrapone observed in the present study under the present experimental condition. Therefore, further study should be made to evaluate role of adrenocorticoids in NST.

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CALORIGENIC RESPONSIVENESS TO ADRENOCORTICAL HORMONES IN COLD-ACCLIMATED RATS. DOI,K.# and KUROSHIMA, A. # Dept. of Physiology, Yamagata University School of Medicine, Nishinomae, Yamagata 990-23 , Dept. of Physiology, Asahikawa Med. Coll., Nishikagura, Asahikawa 078-11

From this laboratory glucagon has been reported to serve thermoregulatory nonshivering thermogenesis through its action on brown adipose tissue (Life Sci., 1982). In the present study calorigenic effect of adrenocortical hormones on whole body oxygen consumption ($\dot{V}o_2$), and interscapular brown adipose tissue (IBAT) and body temperature was investigated in unanesthetized and unrestrained warm-acclimated rats (25°C)(WA)and cold-acclimated ones (5°C)(CA). $\dot{V}o_2$ was measured by using an automatic and manometric apparatus. IBAT and body temperature was continuously measured by means of telemetric transmitter system. Injections of Metopirone (20mg/100g,ip), which causes compensatory increases of desoxycorticosteroids such as desoxycorticosterone or desoxycortisol, and desoxycorticosterone (0.5mg/100g,sc) produced markedly greater elevations of $\dot{V}o_2$, and IBAT and body temperature in CA than in WA. Calculated temperature from increased $\dot{V}o_2$ due to these hormones was highly correlated with the increment of BAT temperature and that of body temperature, respectively. These findings would indicate that the calorigenic responsiveness to adrenocortical hormones is potentiated by cold acclimation and these hormones are involved in the regulation of nonshivering thermogenesis in CA.

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MODIFICATION OF PANCREATIC HORMONE RESPONSES TO NOREPINEPHRINE IN THERMAL ACCLIMATED RATS. OHNO, T., KUROSHIMA, A. and HABARA, Y. Division of Nutrition Physiology, Hokkaido Educational College, Hokumoncho 9, Asahikawa 070 and Dept. of Physiology, Asahikawa Medical College, Nishikagura, Asahikawa 078-11

Effects of norepinephrine (NE) on the portal and aortic pancreatic hormone concentrations were studied in the thermal acclimated rats in order to know role of these hormones in temperature acclimation. NE infusion (2 μg/min, iv, 30 min) under anesthesia effected greater elevation of rectal temperature in the cold-acclimated rat (CA) than in the warm control (WC), while it did not influence the rectal temperature of heat-acclimated rat (HA). Portal and aortic glucagon levels were elevated in the NE -infused CA and WC, but any change was not observed in HA. NE, however, did not affect the portal and aortic insulin levels in CA, but increased the aortic insulin level in HA. Aortic glycerol and free fatty acid (FFA) levels increased in all NE-infused groups. Peripheral FFA level increased in the NE-infused WC, but did not in the NE-infused CA and HA.

These results, taken in toto, suggest that cold acclimation potentiates a glucagon -releasing action of NE and NE-released glucagon cooperate with NE to enhance non-shivering thermogenesis in the cold.

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MODULATION OF INSULIN RECEPTOR OF BROWN ADIPOCYTE DUE TO COLD ACCLIMATION IN RAT. HABARA, Y. and KUROSHIMA, A. Department of Physiology, Asahikawa Medical College, Nishikagura, Asahikawa 078-11

Changes in characteristics of insulin receptor of isolated brown adipocyte were studied in warm-control (25°C,4-5 wks,WC) and cold-acclimated (5°C,4-5 wks,CA) rats using radioactive insulin. Insulin binding against its specific receptor of brown adipocyte was increased in proportion to the number of cells. Binding capacity was affected by incubation temperature. More stable and higher binding was obtained at 25°C than at 37°C. Total binding at 25°C increased during the incubation period in both WC and CA. Total binding was significantly higher in CA than in WC in doses ranging from 0.28 to 7ng/ml. The maximal specific binding was obtained at 5 min of incubation in WC, while the binding tended to increase still after 45 min of incubation in CA. The specific insulin binding was significantly higher in CA than in WC over the experimental period of 45 min. Scatchard plot showed that the number of insulin binding sites was increased to about three times of WC in cold-acclimated brown adipocyte.

These results suggest that insulin is colsely associated with thermogenic function of brown adipose tissue, possibly through its lipogenic action.

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REGULATORY MECHANISMS OF BROWN ADIPOSE TISSUE FUNCTION USING THE TISSUE CULTURE METHOD.
YAHATA,T. AND KUROSHIMA, A. Dept. of Physiology, Asahikawa Medical College,Nishikagura, Asahikawa 078-11

In order to clarify the thermoregulatory mechanisms of brown adipose tissue (BAT), effects of insulin, insulin + glucagon and insulin + noradrenaline on this tissue were directly investigated with BAT from neonatal rat cultured in medium 199 with 10 % featal bovine serum in the humidified 95 % air - 5 % CO_2 at 33 C for 1 week. Insulin stimulated the accumulation of lipids in fat cells and fat cells became larger than control. Addition of glucagon or noradrenaline suppressed the insulin-induced lipid accumulation, but the size of fat cells was not affected. Marked enlargements of nucleus and cytoplasma were observed in the glucagon- or noradrenaline-treated fat cells. Mitochondria of control fat cells showed round or elongated forms and had prominent cristae. Mitochondria of insulin-treated fat cells were round and smaller than those in control cells. Their cristae were not prominent. Glucagon + insulin caused slight, but significant increase in the mitochondrial size. In both insulin- and insulin + glucagon- treated cells, the area of cytoplasma stuffed with small vesicles were observed. These results suggest that insulin stimulates the cytogenesis of brown adipocytes, while glucagon as well as noradrenaline activates the functoin of brown adipocytes.

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ROLES OF CNS ENDORPHIN IN THE THERMOREGULATION.
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Following injection of β-endorphin into the third ventricle (IVR-EP) in the warm-acclimated (at 23°C) adult female rats, a rise in rectal temperature (Tre) was induced during exposure to either warm or hot (33°C) environment, while a fall of Tre under acute cold exposure (3.5°C). "Critical ambient temperature (Ta)" differentiating the rising and lowering responses was estimated to be 5-10°C, but shifted to a higher Ta after acclimatization to hot and to a lower Ta after cold acclimatization. Then, effects of IVR-EP on encephalic, retroperitoneal, subcutaneous, fur surface and tail skin T were examined. A rising effect of IVR-EP in warm environment and a lowering effect of EP under cold exposure were observed exclusively in core T. Under these conditions, IVR-EP did not induce any significant change in surface T, which was attributable to EP-effect on core T. The changes of core T may be due to depression of metabolic thermoregulatory responses. Under the exposure to hot environment, a rise of core T induced by EP was observed with a delay in rising of tail skin T, indicating a suppression of both metabolic and vasomotor responses. Following injection of IVR-naloxone, core T was maintained more precisely than control rats given saline under any conditions. The results suggest that EP may act to suppress the thermoregulatory responses being predominant under each thermal condition by inhibiting the receptivity to thermal input in the CNS, and to prevent the organism from exhaustive reactions to the stress.

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CIRCADIAN LOCOMOTOR ACTIVITY RHYTHM IN THE DJUNGARIAN HAMSTER, PHODOPUS SUNGORUS, UNDER THE INFLUENCES OF TEMPERATURE CYCLE.
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Effects of temperature cycle(25°C during light and 10°C during dark) on circadian locomotor activity rhythm entrained by LD 12:12 were studied in the night active Djungarian hamster. Phase angle differences between activity onset and light off were always more positive and the amounts of activity per day were significantly greater under the conditions with the temperature cycle in comparison with those under constant temperature(25°C). In some individuals, the temperature cycle induced a splitting of the freerunning circadian rhythm in constant dark(DD) into two components, i.e., activity onset component and activity end component. The activity onset component continued to freerun without disturbabces by the cycle, but the activity end component seemed to be entrained by the cycle. In other individuals, splitting did not occur, but the freerunning rhythm in DD was either advanced or delayed by the cycle, depending on the relationship bewtween two phases of the circadian rhythm and the temperature cycle("relative coordination").

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OXYGEN CONSUMPTION AND RECTAL TEMPERATURE OF DEER MICE MEASURED AT VARIOUS ALTITUDES AND SEASONS. YANAGIDAIRA,Y., UEDA,G., MOTOYAMA,T., SAKAI,A., and YONEKAWA,M. Dept.of Adapt. Physiology, Shinshu Univ. Sch. Medicine, Asahi, Matumoto, 390

Wild deer mice (Apodemus argenteus) were captured at three different altitudes, A (610 - 1,000 m), B (1,900 m), and C (2,400 m) throughout one year. After raising for one week at the captured sites, mice were brought back to the 610 m -level and tested in the climatic chamber. First, the $\dot{V}O_2$ in response to 0 °C-exposure was shown to increase for mice captured at mean temperatures ET lower than 0 °C, whereas the values of mice for the temperatures higher than 0 °C remained unchanged. The coefficients of correlations R between $\dot{V}O_2$ and ET were - 0.92 ($p < 0.01$) for the former and 0.38 for the latter (NS), where N = 101. The values ranged from 5.2 to 8.5 ml/kg·min. Secondly, the rectal temperatures RT was shown to have a correlation R = -0.66 ($p < 0.05$). The mice captured at higher altitudes showed higher RT. The values were from 35 to 38 °C. Thirdly, the dry weight of pelt per body weight tended to increase from 5.5 to 8.0 % in winter. In general, the mortality rate of -10 to 0 °C-captured mice was 0 %, whereas that of 0 to 20 °C - mice was 12.9 %.

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EVALUATION OF PHYSICAL ACTIVITY WITH MEASUREMENT OF BODY ACCELERATION. TAMURA, T., NAKAJIMA, K. and TOGAWA, T. Inst. for Med. & Dent. Eng., Tokyo Med. & Dent. Univ., Chiyoda-ku, Tokyo 101

The integral of the positive acceleration versus time wave forms was defined as physical activity. The device that measured physical activity was developed and the relationship between physical activity and energy expenditure was evaluated. Acceleration wave forms of back were measured with strain gauge type transducer. After amplification and rectification, the positive acceleration wave forms were integrated. When the output of integrator reached reference voltage, the output of the comparator flipped from one saturation limit to the other. This output contained a trigger signal. The physical activity was determined by the total counts of the trigger signals. The physical activity, oxygen uptake/body weight and heart rate were determined in ten young males(19-24 yrs.) who jumped at four jumping cycles. With increasing jumping cycles, acceleration counts distributed 0.12 to 0.52G; oxygen uptake/body weight and heart rate ranges were 3.28-31.15 ml/min·kg and 57-140 beats/min, respectively. The oxygen uptake/body weight had good correlation to physical activity (r = 0.922) and heart rate (r = 0.902). These results indicate that this method is a useful evaluation for objectively measuring the physical activity.

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EFFECTS OF CENTRIFUGAL ACCELERATION ON CARDIOPULMONARY SYSTEM IN LABYRINTHECTOMIZED HAMSTERS. WATANABE, S., SATAKE, H., MIZUNO, Y., URANO, H., SEKIMOTO, Y., HAYASHI, R., and MIYAKE, A. Dept. of Neurophysiology, Institute of Equilibrium Research, Gifu University School of Medicine, Tsukasamachi 40, Gifu 500

In this experiment, we examined differences in the cardiopulmonary responses of the intact and labyrinthectomized hamsters to the centrifugal forces. Hamsters were divided into two groups; one was the intact (nonoperated) and another the labyrinthectomized group. Animals were anaesthetized with Nembutal (5-10 mg/100gbw) and fixed on animal holders. Physiological data (ECG, EEG, EMG, respiration) were recorded on a 7 channels data recorder through telemeter. This paper dealt with changes of the heart and respiratory rates and of the relative volume of ventilation which appeared by the centrifugal forces of 2, 4, 6, 8 and 10 G. Effects of acceleration were different accordingly to the axis of the body through which it acts. The animals of both groups had very high tolerance to -Gx, the prone position, which did not cause so large changes in the heart and respiratory rates upto 10 G. In -Gz, the head downward position, they were also tolerant, but a few animals a suddon fall of the heart and respiratory rates in 8 G were caused, but recovered quickly. The tolerance to +GZ, the head upward position, was very low, so that the intact animals caused frequent suddon falls, but recovered, while labyrinthectomized ones could not restore.

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PHYSIOLOGICAL PROPERTIES OF XENOPUS LAEVIS LIVING IN THE HYPOGRAVIC ENVIRONMENT (2).
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Physiological properties in heart and gastrocnemius (gast) muscles were compared between Xenopus laevis (Xenopus) and Rana catesbeiana (Rana). One group of Xenopus was kept in the room air for 6.5 days with minimal volume of water to keep the skin wet. Water content, glutamic-oxaloacetic transaminase (GOT), creatine phosphokinase (CPK), cytochrome oxidase (cyt ox), lactate dehydrogenase (LDH) and its isozyme patterns were analyzed. The GOT and cyt ox activities in gast were higher in Xenopus than Rana. On the other hand, CPK activity and water content were lower in Xenopus. No difference was observed in gast LDH activity but isozyme patterns were different. Rana had two isozymes near cathode side and Xenopus had six isozymes gradually decreasing toward anode. Following the exposure to room air (760 mmHg), GOT, CPK, and LDH activities in gast increased. And gast cyt ox activity decreased to the same level as Rana. The cyt ox activity in Xenopus heart was less than Rana, but no difference was found in the other enzyme activities. Three LDH isozymes were observed in Rana heart and six isozymes in Xenopus. After the exposure to room air, GOT and CPK activities in Xenopus heart were elevated. No change was seen in other parameters. It was suggested that difference of environment may change the metabolic characteristics of heart and gast muscles in frog.

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EFFECTS OF VARIOUS INTENSITY OF MAGNETIC FIELDS ON THE ALBINO RATS UNDER THE NORMODYNAMIC CONDITION.
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Using rats, high and low magnetic field (HM, 200 Oe and LM, 4 mOe) exposure experiments under normodynamic vivarium terrestrial life (ND), and also, for the comparison, under hypokinetic life (HD) were performed. Several parameters, were measured during 3 or more weeks. The control condition of these particular intensities of the magnetic fields is geomagnetic field (GM, arround of 600 mOe). Results: 1) Body weight in HD suffered severe inhibition in all groups, but the effect to LM and HM groups were more than that of GM group. 2) Resting metabolic rate of LM and HM groups strongly decreased in ND. 3) K^+ and Na^+ excretions have a tendency to decrease in HM group, and increase in LM group in ND. In HD, such relation is inverse. 4) The Ca^{2+} excretion rate in NM during HD decreases very strongly during HD. This is a typical LM effects attenuating action of LM. The relation between magnetic field intensity and urinary excretion rate of Ca^{2+} in the vivarium control and hypodynamic animals can be expressed as the theoretical curvilineal regression line of 2 degree, and be able to suggest the reversion of the relation of the Ca^{2+} excretion rate between HD and ND at the special intensity of magnetic fields.

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THE RELATIONSHIP BETWEEN CIRCADIAN VARIATION IN BODY TEMPERATURE AND COMFORTABLE AMBIENT TEMPERATURE. TERAI, Y., ASAYAMA, M., SUGENOYA, J., MIYAGAWA, T., FUJIMATSU, H. and OGAWA, T. Dept. of Phisiol., Aichi Med. Univ., Nagakute, Aichi

The process of circadian variation in body temperature was investigated by examining changes in comfortable ambient temperature selected by the subject during rising, plateau and falling phases of body temperature. Tre and Tty were significantly higher in the plateau phase than in others and similar in the rising and the falling phases. Preferred ambient temperature (Ta-pref) with 40% rh ranged between 28°c and 31°c, where $\bar{T}s$ was approximately 33°c. Ta-pref tended to be higher in the rising phase than in others, but the differences were not significant. Tre minus Ta-pref and Tty minus Ta-pref had the minimum value in the rising phase and the maximum value in the plateau phase. The rate of changes in Tre ($\Delta Tre/\Delta t$) and that in Tty ($\Delta Tty/\Delta t$) in comfortable ambient condition were significantly lower than the rate of change in Tor ($\Delta Tor/\Delta t$) in natural condition. Ta-pref was linearly related to $\Delta Tre/\Delta t$, while more significant negative correlation was noted between $\bar{T}s$ minus Ta-pref and $\Delta Tre/\Delta t$. The results reveal that Ta-pref was affected in relation to $\bar{T}s$ by circadian variations of body temperature.

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EFFECTS OF CLOTHING UPON TEMPERATURE REGULATION IN WOMEN DURING EXERCISE AND RECOVERY AT AMBIENT TEMPERATURE OF 16°C. YAMASHITA, Y. and TOKURA, H. Lab. of Physiol., Dept. of Clothing Sci., Nara Women's University. Nara 630

The effects of fabric moisture and water transport properties on temperature regulation in women during both 30 min exercise (78 W) and 30 min recovery periods at ambient temperature of 16°C were studied. We found the fall of rectal temperatures significantly smaller, but that of mean skin temperatures and that of temperatures of clothing microclimate measured between back skin surface and the garments significantly greater during 30 min recovery period in the subject having worn the cotton garments(C) having good fibre moisture absorbancy than in the subject having worn the acryl garments(A) and the acryl ones with hydrophile properties(At), which have poor fibre moisture absorbancy. Five out of seven subjects preferred C to A and At as comfortable garments during 30 min recovery period. The reason for such differences occurring was discussed in view of thermal physiology and differences of fabric moisture, water and heat transport properties.

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EFFECTS OF HEAT LOAD AND INTENSITY OF WORK ON PATTERNS OF LOCAL SWEAT DISTRIBUTION. TSUJITA, J., TOHORI, M., HORI, S., *KIMURA, M. and *ARAKI, T. Dept. of Physiol., Hyogo Coll. of Med., Nishinomiya, Hyogo 663, *Dept. of Health Science, Hyogo Univ. of Teacher Education, Hyogo 673-14

Observations of regional relationship of the sweat rate at rest and during exercise in cool and hot environments were made on four middle aged male subjects. Exercise was performed on a bicycle ergometer at a constant work load at a cycling rate of 50 rpm for 90 min. The maximal oxygen intake was measured by bicycle ergometer exercise in a room at 25 °C. Work intensity was kept at a level corresponding to 30, 40, 50, 60, 70, 80 % of the maximal oxygen intake in each experiment. Local sweat rates at 19 cutaneous sites were measured successively 15 min intervals by the filter paper method. Total sweat volumes were more closely related to metabolic rate during exercise than rise in core temperature and ratio of oxygen intake during exercise to maximal oxygen intake. There were great individual variations in patterns of local sweat distribution at rest and during exercise. Local sweat rates in the trunk tended to be greater in those in the extremity. The effect of changes in ambient temperature on patterns of local sweat distribution was small. Local sweat rates on the chest, thigh and upper arm in comparison with those on other cutaneous sites were greater in heavier work load than in lighter work load.

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HIDROMEIOSIS DURING EXERCISE IN HOT HUMID ENVIRONMENT. ASAYAMA,M., FUJIMATSU, H., OGAWA, T., SUGENOYA, J., MIYAGAWA, T., TERAI, Y. Dept. of Physiol., Aichi Med. Univ., Nagakute, Aichi 480-11

Hidromeiosis during exercise for 90 min was examind in hot humid environments. Three experimental conditions were imposed on each of 5 male subjects; (1) standard (Ta, 35°C; rh, 75%; 40%$\dot{V}O_2$max), (2) very humid (rh, 85%), (3) heavier work (60%$\dot{V}O_2$max) conditions. Time course of the rate of sweating ($\dot{M}$sw), drippage ($\dot{M}$dr) and evaporation ($\dot{M}$e) measured by the use of a Potter's bed balance and a load cell scale. Rectal and skin temperatures and metabolic rate were also measured. The data were compared with those obtained previously during rest. $\dot{M}$sw increased during the first 20 min and then remained relatively constant for 20-25 min with heavier work and very humid conditions. A decrease in $\dot{M}$sw began in 40-50 min of exercise. It reduced only $\dot{M}$dr, while $\dot{M}$dr remained constant. Sweat drippage occurrd sooner in the exercise than in the rest. The slope of the regression line relating the rate of sweat decline to the maximum dripping rate was steeper during exercise than during rest. The results suggest that accelerated hidromeiosis during exercise contributes to conservation of body fluid.

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TEMPERATURE REGULATION DURING THERMAL TRANSIENT AND EXERCISE AT A SIMULATED HIGH ALTITUDE (380 mmHg). SAGAWA, S., SHIRAKI, K., and KONDA, N. Dept. of Physiol., Sch. of Med., Univ. Occupational and Environmental Health, 807 Kitakyushu

Sweating response in hypoxia is a subject that should be studied for the elucidation of the mechanism underlying the thermal adaptation of man at high altitude. Resting men dressed in shorts were exposed to a high ambient temperature at a simulated altitude of 5,600 m (hypobaric hypoxia) in comparison to that during inhalation of a low oxygen gas mixture (10.5%) at sea level (normobaric hypoxia). Air temperature was raised to 43°C at a constant rate of 1°C/min. Continuous measurements were made of esophageal (Tes) and mean skin ($\bar{T}$sk) temperatures, metabolic heat production, weight loss due to evaporation of sweat, and non-evaporative heat exchange by means of heat flux transducers. Both normo- and hypobaric hypoxia increased $\bar{T}$sk, but reduced Tsk of the distal parts of the body, which indicated that a low oxygen tension would alter the distribution of blood flow. When the pre-exposure Tes at altitude was equal to the pre-exposure Tes at sea level, Tes at onset of sweating induced by the heat exposure was higher at high altitude. Tes at onset of sweating induced by the exercise at high altitude was higher than that at sea level. These experiments may suggest that central drive for sweating is modified by the changes in the distribution of blood flow in a hypoxic environment.

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LOCAL SKIN WETTEDNESS AND THE OCCURRENCE OF HIDROMEIOSIS. SUGENOYA, J., OGAWA, T., ASAYAMA, M., MIYAGAWA, T. and TERAI, Y. Dept. of Physiology, Aichi Medical University, Nagakute-cho, Aichi 480-11

Previously we demonstrated through the ventilating capsule method that hidromeiosis can develop in the absence of drippage of sweat. In this study we examined the relationship between the skin wettedness (W) and the degree of hidromeiosis in a local skin area. In each of 2 cases, 3 capsules fitted on forearm areas were ventilated with different rates of flow (0.2-2 L/min) of air with rh of about 40%. Ta and øa were selected to avoid the sweat drippage from the skin surface inside the capsule. Sweat rate (=evaporative rate) was continuously recorded and the rate of sweat decline was measured; W was calculated using h_e which was determined experimentally. Sweat decline occurred with W above 0.6, and the rate of sweat decline was closely related to W. The present results further confirm that hidromeiosis is a phenomenon directly related to local hydration, which is likely responsible for stenosis of the sweat-duct orifice.

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EFFECTS OF LOCAL Ca^{2+}, Mg^{2+} AND Ca-ANTAGONISTS UPON THERMAL SWEATING IN MAN.
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Effects of intradermally administered Ca^{2+}, Mg^{2+}, verapamil and niphedipine were studied in the forearm of men upon neuro-glandular transmission of central sudorific impulses discharged by exposures to a heat in a hot room. Sweating was recorded continuously with VAISALA hygrometers using sweat capsules(i.d.10mm) and dry N_2-gas flow method. Drugs were given through intradermal indwelling needles and their effects upon neuro-glandular transmission of sudorific impulses were determined and expressed as the time length elapsed from entering the hot room to the first appearance of synchronized sweat discharge waves. It was observed that neuro-glandular transmission of the subthreshold sudorific impulses were remarkably facilitated by local Ca^{2+}(10-20 mM) and significantly inhibited by verapamil(0.1-5 mM). Ca^{2+} and verapamil exerted augumenting and decreasing effects upon local sweat volume, respectively, when they were administered during moderate sweating. Mg^{2+}(10-20 mM) exerted no effects upon transmission as well as sweat volume. With niphedipine(0.3 mM) neither inhibitory effects upon transmission nor decreasing effect upon sweat volume was observed. The mechanism of facilitation of neuro-glandular transmission with intradermal cholinergic as well as adrenergic agonists was discussed in relation to the Ca^{2+} effect.

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EFFECTS OF LOCAL LESIONS OF CEREBRUM ON THE FUNCTION OF SWEAT GLANDS IN FOOTPADS OF RATS.
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Various parts of cerebrum of the rats, which showed spontaneous sweating, were injured by applying electric current of 2 mA for 30 sec. After cerebral injury, spontaneous sweating was examined 4 times for 2 weeks by the iodine-starch method. When the dorsomedial and ventromedial hypothalamic areas were injured bilaterally, spontaneous sweating ceased. Similar cerebral lesions of other parts (e.g., thalamus, septum, posterior hypothalamic area) did not produce the cessation of spontaneous sweating. Two weeks after cerebral injury, the rats were anesthetized with amobarbital-Na to abolish spontaneous sweating and various concentrations of mecholyl were injected subcutaneously into the footpads to examine the excitability of sweat glands of the rats with cerebral lesions. In the rats which showed normal spontaneous sweating, 10^{-10} - 10^{-9} g/ml of mecholyl produced local sweating on the pad where mecholyl was injected. However, in the rats whose spontaneous sweating ceased after cerebral lesions, the palmar and plantar sweat glands showed sweating in response to 10^{-8} - 10^{-4} and 10^{-4} - 10^{-3} g/ml of mecholyl, respectively.

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THRESHOLD DISSOCIATION BETWEEN CUTANEOUS VASOCONSTRICTION AND SHIVERING DURING PYROGEN FEVER.
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It is generally accepted that thermoregulatory reactions are not qualitatively different between febrile and afebrile status. Threshold temperature initiating each regulatory reactions are equally shifted to a higher level, as the set point temperature reset to a higher level during fever. However, we reported previously that threshold body core temperature for cutaneous vasoconstriction (TCTV) and for shivering (TCTS) did not pursue the same course during a certain period of fever induced by intravenous injection of E. coli LPS. TCTV changed always parallel to body temperature increase, but TCTS once decreased 1.5 hr after LPS injection. In the present experiments, this dissociation between TCTV and TCTS were examined during fever caused by intracerebroventricular (i.c.) injection of LPS and PGE2. In case of i.c. injection of LPS, the same dissociation between TCTV and TCTS were observed: TCTS did not change 2 hr after LPS injection. During PGE2 induced fever, this dissociation was not apparent; both TCTV and TCTS increased as body temperature raised. A possibility was then provided that threshod dissociation between TCTV and TCTS was due to differences between acting site of LPS and PGE2 in fever genesis.

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INCORPORATION OF LABELLED 2-DEOXYGLUCOSE INTO BRAIN REGIONS OF RATS DURING FEVER. TAKASE,Y., MORIMOTO,A., SAKATA,Y. and MURAKAMI,N.
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With [^{14}C]-deoxyglucose ([^{14}C]-DG) we investigated the metabolic activity (MA) of the brainstem of the rat in rising phase of fever induced by endogenous pyrogen (EP). EP 1.5 µl derived from rabbit's blood was microinjected into the preoptic area (POA) and [^{14}C]-DG 10 µCi/100 µg was simultaneously given through the catheter previously inserted into the superior caval vein. After 45 min elapsed 0.9°C increase in rectal temperature was seen and decapitation was done to make brain autoradiogram. From the autoradiogram only in the medial part of ventral thalamus (mVE) increased the [^{14}C]-DG uptake during the rising phase of the fever, but there were no characteristic changes of MA in other regions of brainstem. So we designed to make more potent fever to clear the MA in fever and used prostaglandin E_2 (PGE_2) believed as a mediator of fever. PGE_2 1 µg injection into the POA induced prominent increase of body temperature by 2.0°C 45 min later, and the autoradiogram was examined. In this case there were also no significant changes of MA of brain regions except mVE, but the MA of mVE was higher than in EP-induced fever.

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THERMOREGULATION DURING PLATEAU PHASE OF FEVER IN GUINEA-PIGS. HORI, Y., NAKAYAMA, T., TAMAKI, Y* and KANOSUE, K. Dept of Physiology, Osaka Univ. Med. Sch., Kita-ku, Osaka 530, *Dept of Home Economics, Kenmei Women's Junior College, Hon-machi, Himezi 670

It is generally accepted that autonomic thermoregulatory responses are well maintained during the plateau phase of fever as in afebrile state. In the present study, behavioral and autonomic thermoregulatory responses to changes in preoptic and anterior hypothalamic temperature (T_{hy}) were observed in guinea-pigs in afebrile and febrile states. The animals were trained to receive a 5°C cold air for 5 sec by a bar-press at load air temperatures (T_{load}) of 38 and 40°C. Fever was produced by i.m. injection of endotoxin derived from E. coli. As a summed index of the behavioral and autonomic thermoregulatory responses, we used the difference between the ear (T_{ear}) and the selected ambient ($T_{a.sel}$) temperature. The difference ($T_{ear} - T_{a.sel}$) was smaller in the plateau phase than that in the afebrile state at T_{load} of 38°C. The ($T_{ear} - T_{a.sel}$) was linearly related to T_{hy} changes in the afebrile states. The linear relation was also observed in the plateau phases. There was no difference in the rate of increase in ($T_{ear} - T_{a.sel}$) per 1°C rise in T_{hy} between the afebrile and the plateau phase, while ($T_{ear} - T_{a.sel}$) was smaller at the same T_{hy} in the plateau phase at T_{load} of 38 and 40°C.

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BRAIN REGIONS WITH INCREASED INCORPORATION OF [^{14}C]-DEOXYGLUCOSE DURING FEEDING BEHAVIOR. MORIMOTO,A., ONO,T., YANASE,M. and MURAKAMI,N.
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The central nervous structures involved in bar-pressing movement and feeding movement during operant behavior of food intake were investigated in rats by the method of [^{14}C]-deoxyglucose ([^{14}C]-DG) autoradiography. In the present experiment, six animals were divided into 3 groups as follows: (1) FR-1 (174 ps, 160 ps), n=2: each rat ate a food-pellet (40 mg) per one bar-press and received total of 174 pieces (ps) or 160 ps foods. (2) FR-0 (162 ps, 155 ps), n=2: each rat ate one pellet expelled automatically every 15 second and received total of 162 ps or 155 ps foods. (3) FR-0 (68 ps, 56 ps), n=2: each rat ate one pellet expelled automatically every 40 second and received total of 68 ps or 56 ps foods. During bar-pressing movement, the [^{14}C]-DG incorporation increased in the sensory motor cortex, ventromedial thalamus, pars reticulata of the substantia nigra and the medullary reticular formation. On the other hand, an increase of the [^{14}C]-DG incorporation induced by feeding movement was observed in the fornix, parietal cortex, trigeminal nuclei and the solitary nucleus.

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UNIT ACTIVITY IN THE MONKEY ORBITOFRONTAL CORTEX DURING THERMOREGULATORY AND FEEDING BEHAVIORS. HORI, T., KIYOHARA, T., OOMURA, Y.†, NISHINO, H.†and AOU, S.† Dept. of Physiol., Saga Medical College, Saga 840-01 and †Dept. of Humoral Control, National Inst. Physiol. Sci., Okazaki 444.

Single unit activities were recorded from the orbitofrontal cortex (OBF) during high FR bar press tasks for seeking cool air or food in the monkey. The monkey performed the cooling task under a guidance of a cue (green) lamp during heat exposure (Ta, 40-45°C) and was rewarded with cool (22-25°C) air. Feeding task was guided by a red lamp. In a total of 63 units, 29 (46%) units and 18 (28.5%) units showed sustained decrease and increase in activities respectively during bar press or during the period from bar press to reward acquisition in the cooling task, but not during a similar rise and fall in temperature with no bar pressing. Two units responded phasically when the cue lamp was off in the reward period. The majority of the units responding during cooling task showed similar types of responses during feeding task. However, 14 of 18 units showing excitation during cooling task exhibited a peculiar pattern of response which was different from those during feeding task. Their activities increased gradually during bar press and reached a maximum after reward presentation. Task related activities of OBF units were liable to change with the reward situation and motivation in both types of behaviors. Possible roles of OBF neurons in the control of goal-directed behaviors were discussed.

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EFFECT OF NORADRENALINE AND SEROTONIN ON THE THERMO-SENSITIVE NEURONS IN SLICE OF RAT HYPOTHALAMUS AND MEDULLA OBLONGATA. WATANABE,T., ONO,T. and MURAKAMI,N. Dept. of Physiol., Yamaguchi Univ. School of Med., Ube, Yamaguchi 755.

With extracellular recording, we investigated the thermo-sensitive neurons in slices of the preoptic/anterior hypothalamic region (PO/AH) and the medial vestibular nucleus (NVM) of rats, and examined the effect of NA or 5-HT added to perfusate on the neurons. 1) In the PO/AH, most of the warm-sensitive neurons (W-S) (10/11) increased their firing rates with application of 5-HT, while 11 of 13 W-S neurons decreased with NA. These results follow a line of the Feldberg-Myers hypothesis. (2) In the NVM, 11 of 12 W-S neurons behaved the same way as those in the PO/AH to application of 5-HT and could not find a consistent effect with NA. (3) Calculating cumulative log concentration-response curves, we analized the amine sensitivity in the thermo-sensitive neurons. A parallel shift in the concentration-response curve of 5-HT or NA of the W-S neurons was observed between in the NVM and in the PO/AH.

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THE EFFECT OF IONTOPHORETICALLY APPLIED ANGIOTENSIN II AND VASOPRESSIN ON PREOPTIC THERMOSENSITIVE NEURONS IN THE RAT. KIYOHARA, T., HORI, T., SHIBATA, M. and NAKASHIMA, T. Department of Physiology, Saga Medical College, Saga 840-01

Single-unit activities were recorded with multibarrelled microelectrodes from medial preoptic area (MPO) of urethane-anesthetized rats. Thermosensitivity of neurons recorded was examined by changing the local hypothalamic temperature through the implanted water-perfusing thermode. Subsequently, Angiotensin II (AII), Saralasin and Arginine vasopressin (AVP) were applied iontophoretically to the recording cells.

A total of 112 MPO neurons were studied for their responses to thermal and pharmacological (AII) stimulation. Of these, 37 neurons were warm-units and 13 neurons were cold-units. The remaining 62 neurons were thermally insensitive. An iontophoretic application of AII excited 17 of 37 warm-units, inhibited 10 warm-units and had no effect on 10 warm-units. Of 13 cold-units, 8 units were inhibited and 2 were excited by AII. Simultaneous application of Saralasin could reversibly block the AII induced excitation of warm-units. Of 62 thermally insensitive neurons, AII had no effect on 45 units.

The effect of AVP was studied on 53 MPO neurons. AVP induced excitation in 33.3% of warm-units (5 of 15) and inhibition in 46.7% of warm-units (7 of 15). Almost all (5 of 6) cold-units were inhibited by AVP. In 24 of 32 thermally insensitive neurons, AVP had no effect.

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THERMOSENSITIVITY AND OSMOSENSITIVITY ON PREOPTIC NEURONS IN HYPOTHALAMIC SLICES IN VITRO. NAKASHIMA, T., HORI, T., KIYOHARA, T. and SHIBATA, M. Dept. of Physiology, Saga Medical College, Saga 840-01

Several evidences suggest that there is considerable interference between the thermoregulation control system and the osmoregulation control system. In order to understand the possible central linkage between these two systems, it is important to determine whether preoptic thermosensitive neurons respond to local osmotic changes. To answer this, we have investigated the effects of local osmotic changes on activities of thermosensitive neurons in rat's preoptic slices in vitro.

A total of 60 single-units were studied for their responsiveness to both changes in slice temperature and perfusate osmolality. Of these, 17 warm-units, 2 cold-units and 2 thermally insensitive units increased the firing rates to a decrease in osmolality by 15 mOsm/kg. Seven warm-units and one cold-unit were excited by increased osmolality of 15 mOsm/kg. The remaining 31 units did not respond to osmotic changes between 285 and 315 mOsm/kg. Some preoptic warm-units did not lose both the thermosensitivity and the osmosensitivity in the Ca^{2+} deficient medium. The results indicate that some of preoptic thermosensitive neurons in vitro respond to changes in osmotic pressure of the surrouding medium.

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Responses in the dorsal horn neurons to thermal and mechanical stimulation of the scrotum, tail and hind pow in rats. Yamasato, T. and Pierau, Fr. -K.* Department of Physiology, Okayama University Medical School, Okayama, 700.*Max Planck Institut, Bad Nauheim, West Germany.

Spike potentials of the dorsal horn neurons in response to thermal and mechanical stimulation of the scrotum, tail and hind pow were recorded at the 6th lumber and first sacral segments.

Warm stimulation of the scrotum increased spontaneous activity of 31 neurons(warm reactive) and decreased that of 8 neurons(inverse warm). Cold stimulation also induced increased activity of 14 neurons and decreased activity of 2 neurons. Sometimes, double innervation neurons which responsed to both warm and cold stimulation were found in lamina IV, V, VII and X.

No response to mechanical stimulation was recorded from 124(39 %) of 317 neurons and 193 neurons(61 %) responsed to mechanical stimulation. About 17 % of former neurons responsed to thermal stimulation, while 21 % of latter neurons did to thermal stimulation. Thermal sensitive neurons were located in lamina I - V, VII and X. No different irritability to thermal stimulation was found between mechano-sensitive and -insensitive neurons

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INFLUENCES OF SCROTAL THERMAL STIMULATION ON THE VENTROMEDIAL HYPOTHALAMIC NEURONS IN RATS. IMAI-MATSUMURA, K., NAKAYAMA, T. and MATSUMURA, K. Dept of Physiol., Osaka Univ. Med. Sch., Kita-ku Osaka, 530

The effects of scrotal thermal stimulation (SC stimulation) and preoptic thermal stimulation (PO stimulation) were observed on neurons of the ventromedial hypothalamus (VMH) in rats. Fourteen neurons out of 15 which were facilitated by iontophoretically applied glucose responded to SC stimulation. Out of 14, 9 were facilitated by warming. In comparison, 26 neurons out of 52 which did not respond to glucose were not influenced by SC stimulation, but 18 were inhibited by warming. In most cases, neurons facilitated by SC warming were facilitated by PO warming, those inhibited by SC warming were inhibited by PO warming and irresponsive neurons to SC stimulation did not respond to PO stimulation.

Compared with the results observed in the neurons of the lateral hypothalamus (LH), most of glucose responsive neurons in LH and VMH responded to SC stimulation but the responses of LH and VMH neurons to SC stimulation showed reciprocity. These results suggest that the thermal signals evoked by SC and PO stimulation are transmitted to LH and VMH neurons and influence the feeding control.

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RESPONSES OF THE THALAMIC AND HYPOTHALAMIC NEURONS TO SCROTAL WARMING AND NOXIOUS STIMULATIONS IN RATS. KANOSUE, K., NAKAYAMA, T. and ISHIKAWA, Y. Dept of Physiology, Osaka Univ. Med. Sch., Kita-ku, Osaka 530

Neurons in the rat's thalamus and hypothalamus abruptly change their firing rates from minimum to maximum, or vice versa, with increase in scrotal temperature of only 2°C or less ("switching response"). The threshold scrotal temperatures, which were observed on different occasions in many rats, have been reported to be in a range of 32 to 40°C. When observed on 2 neurons simultaneously recorded, however, the threshold scrotal temperatures coincided in all 19 pairs of neurons recorded from the right and left thalamus and in all 20 pairs from the right thalamus and left hypothalamus. These results indicate that the thalamic and hypothalamic neurons change their activities all at once at the same threshold temperature.

A close correlation was observed between neuronal responses to scrotal warming and those to various noxious stimulations. Almost all the neurons excited and inhibited by scrotal warming were also excited and inhibited by noxious stimulations, respectively. The responses to both scrotal warming and noxious stimulation always accompanied the desynchronization of cortical EEG. The desynchronization was observed at about the same time as the neuronal switching responses.

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STUDY ON TEMPERATURE REGULATION IN PO/AH IMPAIRED RABBITS. KOSAKA, M., OHWATARI, N., FUJIWARA, M., INOMOTO, T. AND TSUCHIYA, K. Dept. of Environment. Physiol. and Epidemiol., Inst. for Tropical Medicine, Nagasaki Univ., Sakamoto-machi, Nagasaki

Unanesthetized PO/AH impaired rabbits were exposed to general heat (Ta=40°C) and cold (Ta=15°C) environment, respectively. Temperatures of rectum, PO/AH, reticular formation and ear skin were continuously measured with thermocouples. Respiratory frequency was detected from the temperature changes between the inspired and expired air at the nasal cavity. Although hypothalamic blood flows and metabolic activities observed in intact (control) rabbits diminished or vanished in PO/AH impaired rabbits, the time courses of the changes in various autonomic thermoregulatory responses induced by general heating and cooling of the skin in these PO/AH impaired rabbits closely resembled those observed in PO/AH intact (control) animals. In the PO/AH impaired heat-acclimated rabbits (the former), rectal temperatures were always detected 0.3-0.4°C higher, and the threshold temperature for eliciting those heat loss responses also shifted to higher level as compared with that in the PO/AH impaired cold-acclimated rabbits (the latter). Gains of evaporative heat loss functions to general heating increased quantitatively in the former compared with those in the latter. These results suggest that the temperature regulation system and thermal acclimation must be composed of hierarchically organized control loops located in thermosensitive tissues of central nervous axis.

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EFFECTS OF LSD AND PCPA-PRETREATMENT ON CUTANEOUS AND RENAL VASCULAR RESPONSRS ELICITED BY SPINAL CORD WARMING IN THE RABBIT. NAGAI, M. and IRIKI, M. Dept. Physiol., Med. Univ. Yamanashi, Tamaho, Nakakoma, Yamanashi 409-38

Participation of serotonergic system in thermoregulatory responses elicited by thermal stimulation of the spinal cord was examined in the rabbit.

d-Lysergic acid diethylamide (LSD) applied intraperitoneally at a dose of 40 - 50 μg/kg, suppressed vasodilatation of the ear skin during spinal cord warming. This indicates that brain serotonergic system participates in establishing cutaneous vasodilatation induced by temperature signals arising from the spinal cord.

In serotonin-deprived rabbits with p-chlorophenylalanine (PCPA, 300 mg/kg and 150 mg/kg i.p., daily), dilatatory response of the ear skin and constrictor response of the renal vasculature during spinal cord warming were intensified.

LSD and PCPA-pretreatment did not affect the pressor response to unilateral common carotid occlusion. This indicates that LSD and PCPA-pretreatment did not act generally on the efferent sympathetic activities, but rather specifically on the vascular responses elicited by spinal cord warming.

The present results showed that brain serotonergic system participates in thermoregulatory vascular responses induced by spinal cord temperature stimulation.

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EFFECTS OF SPINAL CORD COOLING ON THERMOREGULATORY RESPONSES DURING EXERCISE IN THE PIGEON. NOMOTO, S., NOMOTO-KOZAWA, E. and IRIKI, M. *
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* Dept. Physiol., Med. Univ. Yamanashi, Nakakoma-gun, Yamanashi-409-38

It has been reported that shivering may occur during exercise in human subjects (Hong & Nadel, 1979). The present study was designed to investigate the effects of spinal cooling on thermoregulatory responses during exercise in the pigeon.

Six domestic pigeons with chronically implanted spinal thermodes were exercised on a treadmill for 15 min at a speed of 0.6 km/h at neutral ambient temperature. Oxygen uptake ($\dot{V}o_2$), respiratory frequency (RF), spinal temp. (Tsc), rectal temp.(Tr), and skin temps. of back(Ts-b) and foot(Ts-f) were continuously measured. During exercise the spinal cord was cooled to 34.7 ± 0.4 ℃ (mean ± SE) for 5 min.

$\dot{V}o_2$, RF and Tr increased by 2.2 times, 1.9 times and 0.3 ℃, respectively, during exercise before cooling. Spinal cooling induced further increase in $\dot{V}o_2$, RF and Tr (3.0 times, 2.1 times the resting values, 0.4 ℃ higher than before cooling). Skin temps.(Ts-b, Ts-f) decreased by 0.2 and 0.7 ℃ during spinal cooling.

The results indicate that cooling of the spinal cord elicits shivering and peripheral vasoconstriction in the exercising pigeon, showing higher Tr than resting one, at thermoneutral conditions.

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EFFECTS OF MENSTRUAL CYCLE ON CUTANEOUS VASCULAR TONE DURING EXERCISE. HIRATA, K. and NAGASAKA, T. Dept. of Physiol., School of Med., Kanazawa Univ., Kanazawa 920

The effects of menstrual cycle on dry heat loss from the hand ($R_h + C_h$), finger blood flow (FBF) and finger venous volume (FVV) were studied in exercising females. Seven healthy subjects exercised for 60 min at 35 % of maximum aerobic power ($\dot{V}O_{2\ max}$), and another 4 subjects exercised for 30 min at 35, 45 and 60 % $\dot{V}O_{2\ max}$ at an ambient temperature of 20°C. ($R_h + C_h$) was measured by a direct hand calorimeter, FBF and FVV were measured by venous occlusion plethysmography and a Whitney strain gauge, respectively. The increase in rectal temperature (T_{re}) during exercise for 60 min was greater in the luteal phase (LP) than in the follicular phase (FP) of menstrual cycle. ($R_h + C_h$) increased in proportion to the rise in T_{re} in both phases. The slope of the relationship between ($R_h + C_h$) and T_{re} was significantly lower in LP than in FP. FBF and FVV increased in proportion to the rise in esophageal temperature (T_{es}). The slopes showing the relationship between FBF and T_{es}, FVV and T_{es} in LP and in FP were not significantly different from each other at any exercise intensity. FVV at a given FBF was less in LP than in FP. The reduction of the venous volume in the finger at a given skin blood flow can be partly the cause of the greater rise in T_{re} during exercise in the luteal phase.

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DIGITAL VASCULAR HUNTING REACTIONS TO LOCAL COLD IN AIR AND ICE WATER. WATANUKI, M., IHZUKA, H., HORI, S. and *KADOWAKI, I. Dept. of Physiol., Hyogo Coll. of Med., Nishinomiya, Hyogo 663, *Dept. of Hygiene, Kyoto Prefect. Univ. of Med., Kyoto 602

Fifteen male university students were selected as subjects. In an air-conditioned room of 27 °C with 70 % relative humidity, two series of experiments were performed in winter. In the first series of experiments, left hand was inserted palm downwards and with fingers extended slightly up to the wrist into the cold chamber of -10 °C with wind velocity of 20 cm/sec for 30 min. In the second series of experiments, the left hand was dipped in stirring water of 5 °C for 30 min. In both series of experiments, the skin temperature on the center of the dorsal surface of the distal phalanx of middle finger was recorded continuously by copper-constantan thermocouple. In both series of experiments, the time for first temperature rise and recovery time were shorter as the skin temperature of finger before cold exposure was higher, the temperature of first rise and the mean skin temperature during cold exposure tended to be higher with rise in skin temperature of finger before cold exposure. The mean skin temperature during cold exposure in air correlated fairly well with that in ice water.

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MEASUREMENT OF NON-EVAPORATIVE HEAT EXCHANGE BY USING HEAT FLUX TRANSDUCERS AT VARIOUS CONDITIONS.

KONDA, N., SHIRAKI, K. and SAGAWA, S. Dept. of Physiol., Sch. of Med., University of Occupational and Environmental Health, Kitakyushu 807

Healthy males were subjected to measure the non-evaporative heat exchange by using heat flux transducers (HFT) under various physical and environmental conditions. Non-evaporative heat exchange (Qflux) was computed from 15 heat flux measurements. At a neutral temperature, metabolic heat production (M) equaled the sum of Qflux and evaporative heat loss (E). When non-evaporative heat exchange at higher ambient temperature (Ta) (38 and 43°C) was calculated by applying the same heat transfer coefficient (hc) of 28°C, it was much higher than the value reasonably obtained by HFT. The discrepancy may be possibly explained as follows; the effective Ta at the surface of the skin will be lower than the average Ta to be used for the calculation of convective heat exchange. On the contrary HFT can measure a direct heat exchange between the adjacent ambient to the skin. The use of HFT also gave a satisfactory result to measure the human thermal balance at a simulated high altitude. We could simulate the change of the esophageal temperature during a heat exposure at 43°C, and estimate dynamic changes of heat distribution of core and the shell during exercise by using Qflux, E and M. Above facts may reveal that we can estimate a wide range of thermal homeostasis satisfactorily by the direct measurement of Qflux, E and M.

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DYNAMIC PROPERTY OF THERMOREGULATORY SYSTEM IN YOUNG AND OLD RATS INDUCED BY PERIODIC CHANGE OF AMBIENT TEMPERATURE - II

ASAKI, Y. AND IRIKI, M.* Dept. of Physiol., Tokyo Metrop. Inst. Gerontol., *Dept. of Physiol., Med. Univ. of Yamanashi.

To measure the dynamic property of thermoregulatory system, ambient temperature Ta was periodically and sinusoidaly changed. Mean ambient temperature (mean Ta) during periodic change of Ta were set at between 10 and 35 °C, and periodic intervals were between 0.25 and 4 hours. Young (5-9 months old) and old (24-25 months old) male rats were used with free moving. The rectal, tail skin and ambient temperature (Tr, Ts & Ta) were measured by thermistar thermometer.

In young rats, 1) mean Trs during periodic change of Ta were almost constant between 10 and 30 °C mean Ta, 2) the ratio the change of Tr to the change of Ta (ΔTr/ΔTa) at 30 °C and 35 °C of mean Ta on 1 hour periodic interval were significantly larger than at 20 °C of mean Ta, and 3) ΔTr/ΔTa at 30 °C of mean Ta became larger by increasing periodic interval from 0.02 ± 0.002 (mean ± SE) on 0.25 hr periodic interval to 0.19 ± 0.02 on 4 hr periodic interval. The significant characteristics of old rats compared with that of young rats were that 1) mean Trs on 1 hr periodic interval increased depending on the increase of mean Ta, and that 2) ΔTr/ΔTa at 10 °C of mean Ta on 1 hr periodic interval was larger.

AUTHOR'S INDEX

* 印は非会員を示す

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〔会　報〕

日本生理学会昭和58年度第1回常任幹事会議事録

日　時：昭和58年4月4日(月)　午後2時～7時
会　場：大阪市北区中之島関電会館
出席者：伊藤正男，星　　猛，酒井敏夫，広重　力，加藤正道，田崎京二，本間三郎，高木貞敬，新島　旭，本郷利憲，島津　浩，真島英信，塚田裕三，竹内　昭，古河太郎，入沢　宏，御手洗玄洋，永坂鉄夫，藤本　守，中山沃，及川俊彦，後藤昌義，大村　裕，纐纈教三，菊地鎌二(教育委員長)，岩間吉也，中馬一郎，中山昭雄，河村洋二郎(当番幹事)
欠席者：内薗耕二，久野　宗，鈴木泰三
議　長：中馬一郎，中山昭雄，岩間吉也，河村洋二郎

Ⅰ．報　告

1．庶務報告（伊藤庶務幹事）

◦会員の移動について

昭和58年1月より3月の期間，入会90，退会75があり現在会員総数3,222名(一般会員2,953，評議員920，特別会員25，名誉会員1，準会員243) である．

◦弔　事

寿原健吉評議員および殿村雄治評議員の逝去について報告がなされ，哀悼の意が表された．

◦各財団研究援助の採択について

山田科学振興財団援助Aに，東京医科歯科大学，神野耕太郎教授「膜電位の光学的測定による初期胚における心機能の形成と分化の研究」700万円が採択された．

- Fenn 寄金によるＩＵＰＳシドニー大会参加援助(500ドル) の候補者として，国立生理研，野間昭典助教授を推薦した．
- 第8回バイオメカニズムシンポジウム（昭和58年7月14日～16日）に協賛を求められたので承諾した旨報告され了承された．

2．会計報告（星会計幹事）

日本生理学会昭和57年度決算（昭和57年1月1日より昭和57年12月31日まで）について説明を受け了承された（別表，総会，評議員会議事要旨の項参照)．この決算については，真島，島津両会計監事の監査を受け，承認を得ている旨，報告された．

3．日本生理学雑誌編集報告（酒井編集幹事）

昭和57年度44巻，および昭和58年度45巻1～5号についての発刊と編集状況が報告された．また生理学論文表題集は昨年と同じく，日生誌と切り離して発刊される旨，報告された．

昭和58年度編集委員の交代（4月より）について

菅野富夫，馬詰良樹，品川嘉也の各委員は，黒島晨汎，登坂恒夫，藤本　守の各氏と交代になる旨報告された．

4．J. J. P. 編集，会計報告（中山委員長）

J. J. P. 編集の現状について，最近投稿数がやや減少気味であるため，J. J. P. へ投稿されたいとの希望が述べられた．また，J. J. P. に minireview を掲載する予定である旨報告された．

日本生理学会大会の英文抄録の掲載を日生誌よりJ. J. P. に移す案は，両誌の合同編集委員会で，今後更に検討されることになった．昭和57年度決算と昭和58年度予算の説明があり了承された．(別表，総会，評議員会議事要旨の項参照) 昭和59年度については，購読料を国内会費7,000円(現行) を9,000円に，国内機関15,000円 (現行) を22,000円に，国外購読者80ドル (現行) を100ドルに値上げする予定である旨報告された．

編集委員の半数交代について，昭和58年4月1日より渡辺　昭，高木貞敬両委員の後任として纐纈教三，佐藤昌康両氏が選出され，入沢　宏，中山昭雄両委員は再任され，中山昭雄委員が編集委員長に互選された旨報告された．

5．教育委員会報告（菊地委員長）

生理学教育に関するアンケートの結果は日生誌7月号に掲載予定であること，また補足アンケート調査を今秋行いたい旨述べられた．

第59回日本生理学会総会の解説講演については，現在日生誌に発表されている．また，ビデオテープの申込状況および会計につき報告され，了承された．

6. 研究費委員会報告（御手洗委員長）

科研費に関するアンケート調査を1月26日275機関に発送し，2月28日までに229通の回答を得た。その結果，約70%の人が現状で満足しており，25～30%の人が改定を希望していることが判明した。希望改定項目としては現行の3細目を4細目にすることが挙げられた.

7. 選挙管理委員会報告（島津委員長）

J. J. P. 編集委員会委員の選挙について，昭和58年1月21日評議員に投票依頼，2月12日開票の結果，興奮膜生理一嶺潤教三，感覚生理一佐藤昌康，心臓脈管生理一入沢　宏，環境生理一中山昭雄の各委員が選出された旨報告された.

8. 評議員選考委員会報告（高木委員長）

今年度新評議員として推薦された候補者について56名が適格と判定された旨報告された.

9. 生理学用語委員会報告（酒井委員長）

前回，常任幹事会で，58年1月初校の予定であることが報告されたが，さらに見直しを要する点があること，現在の生理学用語集（医学書院版）との関係から，58年8月末頃第1版が発行される予定であることが報告された.

10. 日本生理学教室史編集委員会報告（酒井委員長）

第60回大阪大会の記念写真ができればそれを入れて完成する。現在申込数428であるが，将来の販売を見越して，600部まで増刷したい旨述べられた。なお，表題は，日本生理学教室史上巻となる.

11. 国際生理科学連合報告（本間委員長）

1986年ＩＵＰＳはカナダで行うことが最終決定された．1989年ＩＵＰＳを日本で開催したい旨，ＩＵＰＳ勝木副会長よりシドニー大会の理事会で提案することになった.

また，国際生理科学連合大会（ＩＵＰＳ）日本開催に関する企画準備委員の1人である久野　宗教授（京大）に対し，国際交流基金より旅費援助をする件につき，伊藤幹事より説明がなされた.

12. 生理科学研究連絡委員会報告（本間委員長）

委員として，新たに久野教授（京大）が加わった.

学術会議の改革について，政府試案と学術会議側の改革案との相違点，および改革に伴う生研連のあり方の変化の可能性につき，説明がなされた.

13. 第61回日本生理学会大会（昭和59年）に関する報告（高木幹事）

第61回大会は群馬大学医学部担当（高木貞敬，三浦光彦，鈴木光雄，平尾武久各当番幹事）で行われる.

期日：昭和59年3月28日より30日まで

場所：県民会館，商工会議所，県立図書館

なお，中山（沃）委員より，今後の大会では旅費申請の関係上3月末を避けてほしい旨の希望が述べられた.

14. 第60回日本生理学会大会（昭和60年）に関する報告（岩間当番幹事）

第60回大会は第21回日本医学会総会との関係上，大阪大学の豊中キャンパスで行われる．1293名の登録，656演題申込で，発表は一般口演のみとし，ポスター発表なし，今回の企画として，10名の講師を招待し，「臨床医学との交流シンポジウム」が行われ，また特別講演としては T. N. Wiesel 教授 (Harvard Med Sch) "Morphological basis of visual cortical function" が行われる．評議員会・総会は2日目午後，懇親会は2日目夕刻に行われる．なお，今回は，プログラムを予稿集より独立させ，ポケットサイズにすると共に字を大きく見やすくしたことなどにつき報告された.

15. その他

すでに名誉会員と決定している J. C. Eccles 氏へ贈呈する推挙状と記念品が伊藤幹事から披露された.

Ⅱ．議　題

1. 昭和57年度第2回常任幹事会議事録を承認した.

2. 昭和58年度予算案について

従来，大会立替返却金を収入の部に入れていたが，昭和58年度より大会援助費として支出の部に入れること，国際交流基金より35万円を京大，久野教授のシドニー大会旅費援助として支出することを認め，昭和58年度予算案を了承した．（別表，総会，評議員会議事要旨の項参照）

3. 評議員推薦について

56名の候補者が適格であるとの評議員選考委員会の判定が承認され，評議員会に提出することになった.（別表，総会，評議員会議事要旨の項参照）

4. 特別会員推薦について

御手洗幹事より伊藤　龍氏の，伊藤幹事より朝比奈一男氏の紹介と推薦説明があり，それぞれ特別会員候補として承認された．前回の昭和57年度第2回常任幹事会で特別会員候補者として推薦された伊藤真次，福

田篤郎両氏と共に，4名を昭和58年度総会に提出し，承認を求めることになった．

5. 日本光生物学協会加入の件

慶応大，村上教授より，日本光生物学協会へ日本生理学会が団体加入してほしい旨の提案が書面にて日本生理学会になされた．この件について，これまでに学協会へ日本生理学会が団体加入した前例がないことなどから，継続審議とし，伊藤幹事と村上教授との間で連絡をとり，再提案を待つことになった．

6. 教育委員会昭和58年度活動計画

1) 教育に関するアンケートについて

補足アンケートを行う件につき，昭和58年度予備費より20万円を支出することを了承した．

2) 生理学実習書改訂版の4月発行予定が了承された．

7. 研究費委員会昭和58年度活動計画

アンケート結果は，約70名の人が現状に賛成であるが，研究費委員会は，審査員数，細目数を増加したらどうかとの提案を前向きに受けとめて，その方向に努力し，学術会議委員にこのアンケート結果を生かしてもらいたい旨説明された．

アンケート結果について，科研費の現状の当否を含め，さらに資料分析を深めるとの案と，細目数を1つ増加する方向に努力してもらうとの案の間で採決が行われ，賛成多数で細目を増加させる方向に研究費委員会が努力することが決定された．

8. 生理学教室史出版の件

当初500部の印刷予定であったが，これらは全て購入される見込みなので将来を考えて600部に増刷する件について酒井編集幹事より説明がなされ，予備費が許す範囲で増刷する件を承認した．

9. 国立生理学研究所と日本生理学会との今後の関係について

生理研と日本生理学会との今後の関係について，生理研設立時の主旨にかんがみ，日本生理学会がとるべき今後の方針が高木幹事より提案された．審議の結果，提案内容について検討するための小委員会を常任幹事会に設けるが，小委員会設置の詳細については伊藤庶務幹事と佐藤昌康氏（都神経研）に一任することとなった．

10. 第62回（昭和60年度）日本生理学会大会に関する件

久留米大で開催を引き受ける旨，纐纈幹事より説明があり，了承された．

11. その他

J.J.P.編集委員会委員を2名増員したいとの編集委員会の希望が中山(昭雄)編集委員長より説明され，総会，評議員会にこの件を報告することが承認された．なお，新委員の分担領域は編集委員会に一任された．編集委員選出の投票は今秋に行われる予定である．

第60回日本生理学会評議員会および総会議事要旨

日　時：昭和58年4月6日(水)午後4時5分～6時10分
会　場：阪大法経合同講義棟
出席者：200名
議　長：中馬一郎，中山昭雄，岩間吉也，河村洋二郎(当番幹事)

議長は開会にあたり，恒例にしたがい，評議員会，総会を並行して行うことを提案し，了承された．

Ⅰ．評議員会・総会報告

1) 庶務報告(伊藤庶務幹事)：現在の会員数，評議員数および特別会員について報告がなされた(表1)．森　信胤特別会員，川上正澄評議員，寿原健吉評議員，殿村雄治評議員の逝去について報告がなされ，哀悼の意が表された．山田科学振興財団Aによる研究援助として，東京医歯大医学部第二生理神野耕太郎教授の研究課題(700万)が採択されたこと，Fenn寄金によるIUPSシドニー大会参加援助候補者に野間昭典教授(国立生理研)を推薦した旨の報告がなされた．

2) 昭和57年度会計決算報告(星会計幹事)：昭和57年度の決算報告（表2）がなされ，承認された．

3) 日本生理学雑誌編集報告(酒井編集幹事)：日本生理学雑誌44巻（昭和57年度）の編集内容は次のとおりである．44巻1～12号，発行回数11回（8，9号合併大会号)，総頁数795(表3)．次いで45巻の編集進行状況につき説明があり，4号5号の編集が完了したとの報告がなされた．また，生理学論文表題集は昨年と同じく日生誌と切り離して発刊される旨報告された．4月1日より編集委員に3名の交代があったとの報告

がなされた.

4) J. J. P.編集会計報告(中山委員長):昭和57年度 J. J. P.編集決算,昭和58年度予算案,刊行状況,J. J. P.編集委員改選の結果などの報告があった(表4,5,6).

J. J. P.編集委員を2名増員したいこと,この秋に2名の増員のための選挙を行いたい旨の希望が出され,承認された.日本生理学会大会の英文抄録の掲載を日生誌から J. J. P.に移管する件については,もう少し検討したい旨の報告があった.

5) 教育委員会報告(菊地委員長):生理学教育に関するアンケートの結果は教育委員会のコメントとともに日生誌7月号に掲載予定であること,補足アンケートを今秋行いたい旨の報告があった.昨年の日本生理学会大会における教育講演を収録したビデオテープは順調に販売されたこと,また本年も再版し,発売したい旨の報告があり,会員各位の協力が求められた.日本生理学会編「生理学実習書」の改訂版が本年4月に出されることになっており,販売に協力されるよう要請があった.

6) 研究費委員会報告(御手洗委員長):本年1月科研費に関するアンケート調査を行い,2月末日までに得た229通の回答については現在整理中であり,いずれ完全な形で報告したい旨の報告がなされた.なお,研究費委員会としては,現行の3細目を4細目にする方向で,今後検討を深める旨の報告があった.

7) 選挙管理委員会報告(島津委員長):本年2月12日J. J. P.編集委員選挙開票により4名を選出したことが報告された.

8) 生理学用語委員会報告(酒井委員長):生理学用語集の出版が遅れている状況とともに,本年秋ごろに発行される予定であることが報告された.

9) 日本生理学教室史編集委員会報告(酒井委員長):第60回大阪大会の記念写真が揃えば完成すること,本年5月中には発送の予定であること,表題を「日本生理学教室史 上巻」とすることなどの報告があった.なお,同書の購入,販売に関し会員各位の協力が求められた.

10) 国際生理科学連合報告(勝木副会長):本年2月に西アフリカ Ivory Coast で Sickle Cell Anemia のワークショップが IUPS の後援で開催され,この会の運営に際し,開発途上国への援助金として会員各位から集められた寄付金が充当された旨の報告があり,本ワークショップに協力のため出席した中馬幹事より謝辞が述べられた.1986年 IUPS はカナダのバンクーバーで行うことが決定されたこと,1989年 IUPS を日本で開催したい旨を本年のシドニー大会の理事会で提案する予定であることなどが報告された.

11) 生理科学研究連絡委員会報告(本間委員長):(i)IUPS 理事会に日本から5名が参画する旨の報告がなされた.(ii) 生理研連主催のシンポジウム「生理学研究の動向」,「生体の神経系と体液性の関連調節」が大村 裕教授を世話人として昭和57年11月26,27日九州大学医学部で開かれたとの報告があった.(iii)学術会議の改革について,政府試案と学術会議改革委員会案との相違点,それにともなう学術会議側の対応策などにつき説明がなされた.(iv)伊藤庶務幹事より,日本への IUPS 誘致のため伊藤,星両幹事と京大・久野教授の3名がシドニーでの IUPS 理事会に出席する予定である旨の追加発言があった.

12) 第61回 (昭和59年) 日本生理学会大会に関する報告(高木幹事):群馬大学医学部高木,三浦,鈴木,平尾各当番幹事により,59年3月28,29,30日の3日間,県民会館,商工会議所,県立図書館にて開催されることが報告された.

13) 第60回 (昭和58年) 日本生理学会大会に関する報告(岩間当番幹事):今日現在出席者1,463名,演題数655題であることなどが報告された.

Ⅱ. 議題

1) 昭和58年度予算案について:星会計幹事より説明があり,評議員会の承認を経て,総会に附議し承認された(表7).

2) 評議員推薦について:評議員選考委員会が提案した新評議員候補者はすべて評議員会において承認された(表8).

3) 名誉会員および特別会員推薦について:H. Grundfest 氏の名誉会員推薦につき,また,伊藤真次氏,朝比奈一男氏,伊藤 龍氏,福田篤郎氏の特別会員推薦につき,それぞれ推薦の辞が述べられた後,評議員会より推薦することが承認され,さらに総会で承認された.

4) 第62回 (昭和60年度) 日本生理学会大会に関する件:第62回日本生理学会大会は久留米大学医学部,纐纈教三,西彰五郎当番幹事のもとで開催されることが報告され,了承された.

総会,評議員会終了後,塚田前当番幹事より今回の当番幹事中馬,中山,岩間,河村各氏に謝辞が述べられた.

表1．日本生理学会庶務報告

(昭和57年12月末現在)

会員	
一般会員	3,002名
特別会員	25名
名誉会員	1名
準会員	
学校図書館	133部 (128件)
研究所書店	117部 (73件)
寄贈及び交換	
外国	20部
国内	15部
合計	3,313

特別会員 (25名)

東 龍太郎	井上 清恒	勝 義孝
勝木 新次	勝木 保次	久保 秀雄
黒津 敏行	斎藤幸一郎	瀬尾愛三郎
高木健太郎	戸塚 武彦	問田 直幹
冨田 恒男	名取 禮二	長嶋 長節
西丸 和義	福田 邦三	福原 武
藤森 聞一	松田幸次郎	三田 俊定
簑島 高	吉井直三郎	吉村 寿人
若林 勲		

表2．日本生理学会昭和57年度決算報告

(自 昭和57年1月1日
至 昭和57年12月31日)

収入

	57年度予算	57年度決算	増減	備考
① 前年度繰越金	4,088,594円	4,088,594円		
② 昭和57年度収入	21,985,000	25,828,927		
(内訳)				
会費	16,675,000	17,707,557	+	
購読料	1,800,000	1,955,450	+	
論文掲載料	1,500,000	1,502,774	+	
広告料	1,100,000	1,170,400	+	12件
会誌分冊売	30,000	41,200	+	15件
預金利子	130,000	126,650	−	第一勧業銀行
日本医学会奨励金	100,000	100,000		昭和57年度
印税	300,000	351,351	+	生理学実習書及び用語集
大会費立替返却	300,000	420,776	+	
雑収入	50,000	72,769	+	ＩＵＰＳ案内
論文表題集	—	2,380,000		
①+② 合計	**26,073,594**	**29,917,521**		

支出

(内訳)	57年度予算	57年度決算	増減	備考
会誌印刷代	11,340,000	11,332,789	−	43巻12号—44巻11号
会誌発送代	2,500,000	2,337,049	−	同上
編集会議費	100,000	108,530	+	日本生理学雑誌
校正料	250,000	246,000	−	同上
原稿査読料	30,000	42,000	+	同上
事務室使用料	2,099,040	2,099,040		12ヶ月
事務室光熱費	270,000	290,001	+	同上

通信費	850,000	833,220	−	
事務費	850,000	839,497	−	
備品	80,000	85,030	+	本棚
会合費	300,000	329,720	+	常任幹事会，委員会
旅費	1,200,000	1,232,250	+	同上
人件費	2,600,000	2,907,185	+	アルバイト含む
職員健康保険	70,000	55,944	−	
職員退職金積立	100,000	100,000		
国際交流基金	250,000	250,000		
論文表題集	—	1,564,706		
雑費	100,000	106,522	+	ＩＵＰＳパンフレット発送，供花
予備費	3,084,554	200,000	−	教育委員会アンケート
合計	**26,073,594**	**24,959,483**		
昭和58年度繰越金総額		4,958,038		
合計		**29,917,521**		

表3．日本生理学雑誌44巻（57年度）編集報告

44巻1～12号（8，9号合併）発行11回

44巻総頁数　795頁

（内訳）	編	頁	43巻総頁数
原著	9	98	754頁
短報	2	8	
総説	4	44	
研究法	1	12	
解説講演	16	50	
口演演題		238	
学会抄録		94	
会報，その他（目次，索引含）		193	
広告		58	

尚，業績表題集は別冊として刊行．

表4．ＪＪＰ 昭和57年度決算および58年度予算　（昭和58年3月8日）

	57年度			58年度	
支出	予算額	決算額(見込)	摘要	予算額	摘要
製作費	12,397,000円	12,489,000円	1,088頁	12,627,000円	1,100頁
審査・英文校閲料	1,628,000	1,512,000		1,683,000	
旅費・会議費	810,000	491,000		644,000	
通信・消耗品費	1,455,000	1,389,000		1,389,000	
人件費	2,060,000	2,060,000		2,060,000	
雑事務費	809,000	809,000		809,000	
送料(外国)	3,600,000	3,076,480		3,489,000	
送料(国内)	2,820,000	2,488,430		2,678,000	
支出合計	**25,579,000**	**24,314,910**		**25,379,000**	

収　　入					
国内会員	6,230,000	5,599,300	842名 @7,000 5%未収	6,160,000	880名 @7,000
国内機関	2,250,000	1,845,000	123名 @15,000	1,950,000	130名 @15,000
外国購読	8,448,000	7,856,800	430名 80$ 40$ @230	8,184,000	460名 40$ 80$ @220
別刷バックナンバー	3,414,000	3,334,600		3,414,000	
文部省助成	5,400,000	5,670,000		5,670,000	
繰越金	15,355	15,355		6,145	
収入合計	**25,757,355**	**24,321,055**		**25,384,145**	
差引繰越	178,355	6,145		5,145	

表5．JJP 刊行状況（Vol. 32, No. 6）刊行部数 1,600部

個人購読	842	975	外国購読	424	437
団体購読	123		外国会員・その他	13	
国内寄贈	10				

表6．JJP 論文投稿状況

年度	4月	5月	6月	7月	8月	9月	10月	11月	12月	1月	2月	3月	計
55	9	11	10	8	12	14	13	10	8	7	15	11	128
56	10	5	6	13	12	9	9	16	5	4	9	10	108
57	13	12	8	8	6	6	10	11	10	8	12	2＋α	106＋α

（昭和58年3月10日現在）

表7．日本生理学会昭和58年度予算

（自 昭和58年1月1日 至 昭和58年12月31日）

	収　　入	備　　考
① 前年度繰越金	4,958,038円	
② 昭和58年度収入	23,955,000	
（内　訳）		備　　考
会費	17,190,000	（会費6,000円×3,128×0.9 臨時会費含む）
購読料	1,800,000	（準会費8,000円×250×0.9）
論文掲載料	1,500,000	
広告料	1,100,000	
会誌分冊売	35,000	
預金利子	130,000	（第一勧業銀行）
日本医学会奨励金	100,000	
印税	350,000	（生理学実習書，用語集）
論文表題集	1,700,000	
雑収入	50,000	
①＋② 合計	**28,913,038**	

	支出	備考
（内訳）		
会紙印刷代	11,714,000	(44巻12号—45巻11号)
会誌発送代	2,500,000	(同上)
編集会議費	120,000	(日本生理学雑誌)
校正料	250,000	(同上)
原稿査読料	45,000	(同上)
大会援助費	500,000	
事務室使用料	2,099,040	(12ヶ月)
事務室光熱費	300,000	(同上)
通信費	850,000	(会誌外通信)
事務費	850,000	(印刷物，事務雑費)
備品	80,000	
会合費	350,000	(常任幹事会，委員会)
旅費	1,300,000	(同上)
人件費	3,000,000	(アルバイト含む)
職員健康保健	125,000	
職員退職金積立	100,000	
論文表題集	1,700,000	
国際交流基金	250,000	
雑費	100,000	
予備費	2,679,998	
合計	**28,913,038**	

表8．日本生理学会新評議員

計56名（五十音順）

氏名	所属，現職
粟生 修司	九州大，医，助手
浅木 恭	都，老人研，研究員
安藤 啓司	神戸大，医，助手
安藤 正昭	広島大，総合科，助手
安藤 正人	名保大，医，助手
井上 洋	岐阜大，医，医員
五十嵐勝朗	弘前大，医，講師
池上 司郎	三菱化成，生命研，研究員
石井 和子	福島医大，講師
稲永 清敏	産業医大，助手
上坂 伸宏	京大，医，助手
恵良 聖一	岐阜大，医，助手
大貫 義人	阪大，医，助手
大森 治紀	東大，医，助手
太田 雄興	久留米大，医，講師
奥平 進之	東邦大，医，講師
加藤 昌克	群大，医，助手
加藤 聖	金沢大，神経研，助手
香山 雪彦	阪大，医，助手
河原 剛一	旭川医大，助手
北村 清吉	神奈川衛生短大，教授
久次米健市	国立循環器病センター，室員
小松 明	東京女子医大，助手
後藤 秀機	岩手医大，助教授
米谷快男児	大分医大，講師
沢井 洋子	日大，医，助手
品川 泰子	京大，医，助手
柴田 政章	佐賀医大，助手
陣内皓之祐	滋賀医大，助教授
菅 世智子	弘前大，医，講師
菅原 清	金沢大，神経研，助手
鈴木 和夫	東海大，医，助手
田畑 満生	名大，農，助手
高橋 清久	都，神経研，研究員
高橋 弥穂	秋田大，医，講師
高林 彰	名大，環境医研，助手
滝川 順子	順大，医，助手

滝森　徹　秋田大，医，助手
立花　政夫　生理研，助手
寺川　進　生理研，助教授
中井　正継　国立循環器病センター，室長
中島　敏博　佐賀医大，助手
野本　茂樹　都，老人研，研究員
葉原　芳昭　旭川医大，医，助手
橋口　利雄　東京医大，講師
樋口　隆　福井医大，助教授
彦坂　興秀　東邦大，医，講師
堀　弥生　阪大，医，助手
本間　信治　富山医薬大，助教授
南山　求　国立循環器病センター，研究員
宮原　郷士　九大，医，助手
村上　新治　北大，医療短大，助教授
八幡　剛浩　旭川医大，助手
山口　賢一　新潟大，医，助手
山本　三幸　筑大，医療短大，講師
渡部　和成　愛知学院大，歯，講師

〔お知らせ〕

日本学術会議第13期会員選挙について

日本学術会議中央選挙管理会委員長

第13期会員選挙は本年11月に実施される予定となっておりましたが，日本学術会議会員選挙規則の臨時特例により，選挙期日（郵便投票の締切日）が変更され，**本年12月19日**になりました．

記

有権者の異動の届出について

有権者は，氏名，本籍，住所（住居表示の変更も含む），勤務機関及び職名，勤務地等のいずれかに異動が生じたとき，または，博士の学位を取得したり，名誉教授の称号を授与された場合には，そのつど，すみやかに「有権者異動届」（同封選挙説明書4ページ様式第3参照）により中央選挙管理会に届出をしてください．これを怠ると有権者の権利を行使できないことがあります．

特に住所の異動については，**本年9月24日**（必着）までに届出があれば，投票用紙を新住所あてに送付しますが，その後に届け出られた場合は旧住所あてに送付されることになりますのでご注意願います．

なお，中央選挙管理会が登録用カード記載の住所に投票用紙を発送しても，郵便局から「転居先不明で配達できません，あて所に尋ねあたりません」などの理由で返戻された場合には，有権者名簿から削除されますからご承知くださるようお願いします．

日本学術会議生理科学研究連絡委員会シンポジウム

テーマ　「病態の生理学的基礎」
とき　11月4日（金）10：00AM～5：00PM
ところ　山梨医科大学臨床講堂
（甲府駅よりタクシーにて約20分）
プログラム
10：00～12：00　座長　本間三郎
体温調節と発熱
山梨医大生理　入来正躬
胃収縮運動とむねやけ
群馬大医療短大　伊藤漸
睡眠時における呼吸調節とその障害の生理学的機序
千葉大医生理　本田良行
12：00～1：30　昼休み
1：30～3：50　座長　塚田裕三
心力学と肥大心
順天大医生理　真島英信
不整脈と心筋イオン流
山梨医大薬理　橋本敬太郎
急性心筋梗塞時の神経性循環調節機構
循環器病センター　二宮石雄
心筋ポンプ運動と心疾患（NMR及び核医学映像法による）
東大医放射線　飯尾正宏
3：50～4：10　コーヒーブレーク
4：10～5：00　座長　星猛，入来正躬
総合討論

第10回（昭和58年度）日産学術研究助成候補推薦要領

1. 助成の趣旨

自然科学を主とする学術の有意義な研究であって，国の助成となるべく重複しないもの（その期間が長期に亘る研究，学際的なグループによって行なわれる研究等）に対し助成を行ない，わが国の基礎学術の向上，進展に寄与しようとするものであります．

2. 助成対象研究分野

助成対象を**資源・エネルギー，環境**の分野の基礎研究，および応用研究とし，本年度は当該分野のうち次のような研究を期待します．

必ずしも実験を伴う研究のみでなく，いわゆるソフトの研究も含みます．ただしその場合は調査研究助成として扱います．

① 開発に伴う環境の改変が生態系に及ぼす影響に関する研究
② 生物指標による人間環境の評価に関する研究
③ 新しい原理に基づく環境の分析方法と制御の方法に関する研究
④ 環境汚染物質の生体作用における複合効果に関する研究
⑤ 病害動物に対する無公害駆除剤の開発，並びに生物学的駆除法の研究
⑥ 環境の自然浄化に関する研究
⑦ 新しい機能材料に関する研究
⑧ 人間—機械系の解析による安全性向上の研究
⑨ エネルギーの貯蔵に関する研究

その他，資源・エネルギー，環境の分野における(イ)海洋の利用，(ロ)林産資源の利用，(ハ)輸送システムなどの研究

3. 助成対象の研究者

貴学（協）会に関する自然科学分野の研究に従事しており，2～4年の期間を要する上記の研究を行なおうとする研究者および研究グループで，その研究成果が学術の進歩，発展に貢献するところが大きいと思われるもの．

4. 研究助成金額と助成件数

(1) 研究助成金額

- 総　額（研究全年度）約　210,000千円
- 1件当り助成金額の上限
  一般研究…… 30,000千円（研究期間2～4年）
  調査研究…… 5,000千円（研究期間　2年）
- 助成金の支払い
  昭和59年度(59/4～60/3)を研究第1年度とし，研究計画に従い年度毎にその所要額を支払います．
  ただし，第1年度の交付額は原則として助成額の1/2以下とします．

(2) 助成件数

調査研究を含めて12件前後を予定しています．

5. 推薦件数

1学（協）会より1～2件とします．

6. 推薦者

学（協）会の代表者とします．

7. 推薦手続

所定の推薦用紙に必要事項を記入し，当財団宛に1部をご送付願います．

8. 推薦締切日

昭和58年11月30日（水）
日本生理学会締切日　昭和58年11月10日

≪推薦書提出先・連絡先≫

(財)日産科学振興財団
〒104 東京都中央区銀座6-17-2
電話 03-543-5597

【本誌編集委員会より】

昭和57年度（1982）論文表題集申込み案内

日生誌5号にて上記表題集の申込みご案内を致しましたが，御入用の方は至急お申込み下さい．

〔編集後記〕

「日生誌」45巻8，9合併号がようやく出来上りました．本号には，第60回日本生理学会大会(大阪大学)の口演抄録と総会議事録が集録されています．なお，本大会の特色としてアピールされたノーベル賞受賞に輝く Wiesel 教授の講演や「臨床医学との交流シンポジウム」などの記録は，次号以降に入ることになります．

この号を手にして感じることは，1) 収録された英文抄録が立派になってきたこと，2) 第59回大会（慶応大学）で試みられた演題制限の撤廃が，本大会でも踏襲されて，全体として演題数がかなり多いことです．

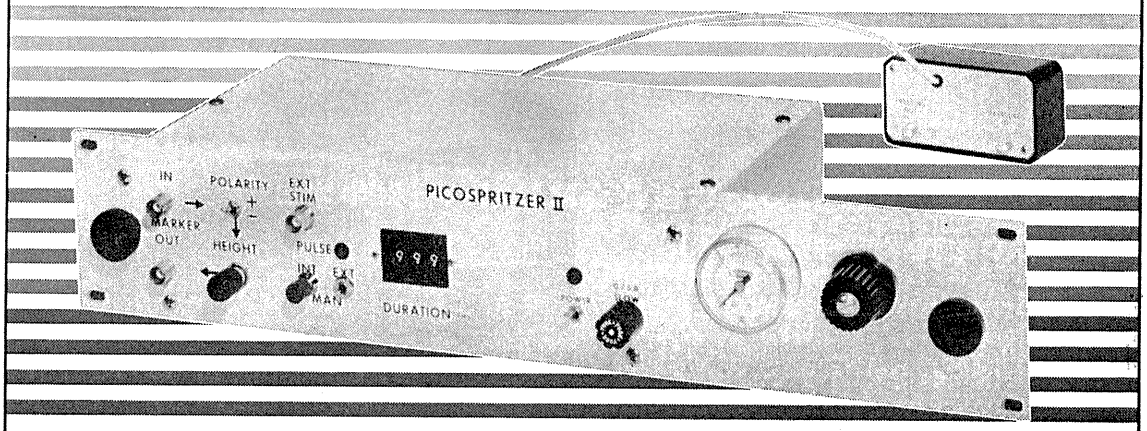
第1回日本生理学会大会が，1922年に東京で誕生して以来，今回の大会は還暦を迎えたことになりますが，当初数十題の演題が，近年には約10倍にもふくれ上り，そのうえ，内容的にも国際レベルをゆくものがふえています．抄録の英文化は，1977年から今日に至るまで継続され，来年の群馬大学の大会でも踏襲されると思いますが，その間，原稿のタイプ印字や印刷技術，さらに英語自体も改善し，このやり方がかなり板についてきた感じです．この英文抄録にワープロによるものが出現する日も近いような気がします．

抄録の英文化は，オリジナルとしての国際的主体性を重んじる意図で進められたと考えますが，それに付随して，最近，学会の正式原著英文誌 JJP に載録することの是非が，両編集委員会で取沙汰されています．しかしながら，日生誌の英文抄録をそのまま JJP に転載または移管することは，現時点ではレフェリーの問題，編集の諸問題が未解決であって，実現はしていません．

本年は，故浦本，内山先生時代から懸案であった日本生理学教室誌（上巻）が40年ぶりに上梓され，実習書，用語集なども次々に完成し，よい年を歴史に刻むことになりましょう．編集委員会では，最近のトピック的総説のほかに，日本生理学会の歴史的な今昔に関する記事をとり上げる予定になっています．皆様の御意見をお待ちしています．　　(藤本　守)

PICOSPRITZER II

圧力吐出に依る細胞内及び細胞外に定量
極微少量(ピコリター単位)試薬押出装置



PICOSPRITZERⅡは標準ラックに取り付ける事が出来ます。
繰り返し連続使用が可能で、吐出量は設定時間と圧力調整に依り任意に変える事が出来ます。
PICOSPRITZERⅡに依る圧力吐出装置はイオン泳動法に依る注入方法に比較して神経組織に対する電気的な影響を心配する必要が全くありません。
本装置は御使用に際し直ちに稼動出来ます様必要な物は全て用意されて居り、亦廉価で経済的に御使用預けます。
PICOSPRITZERにはSingle channel用、multi channel用があります。

■仕　様

電　　源：115 V A.C. 50, 60 Hz
電　　流：1 Amp. max
消費電力：15 watts. max
電 源 コ ー ド：8 feet
操 作 圧 力 範 囲：0-100 PSIG
圧 力 パ ル ス 信 号：2 ms～999 ms
タイムマークシグナル：1 ～30 mv

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待望の〝0.25mm〟

動物用

リージョン・ジェネレータ
MODEL RFG-4A

直径0.25mmのTC電極により、今迄行ないにくかった極めて微少の損傷作成が可能になりました。

- ■Lesion Generatorによる損傷は、小動物の脳組織の損傷に適しており、また手技が極めて簡単です。
- ■いかなる損傷条件(損傷温度、損傷時間)でも生体組織に出血をひきおこすことはありません。
- ■熱センサーによって損傷組織の温度を正確にコントロールすることができ再現性、均一性に優れた損傷巣を作製することができます。
- ■50°C以上の損傷条件では、損傷温度が高ければ高いほど、また損傷時間が長ければ長いほど大きな損傷巣を作製することができます。
- ■外部の刺激装置と本体を接続することにより、同一電極から電気刺激を与えることもできます。

輸入発売元

室町機械株式会社

〒103 東京都中央区日本橋室町4の3(大辻ビル)
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実験動物脳内酵素瞬時不活性化装置

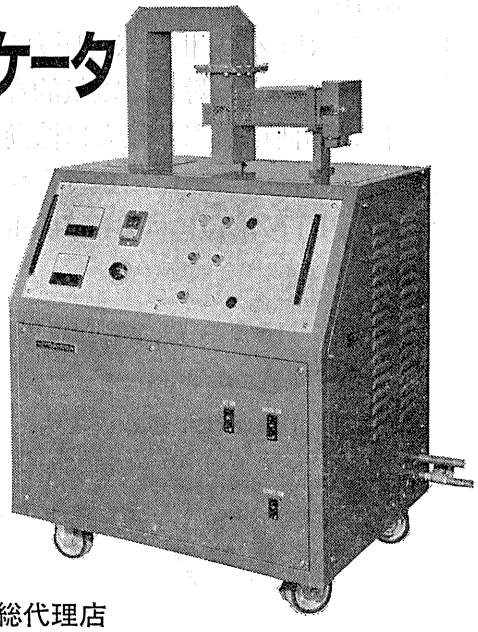
東芝マイクロウェーブアプリケータ
MODEL TMW-6402A

実験動物の脳内物質の測定に先立ち、測定物質に関連する諸酵素を不活性化する方法として凍結法があります。しかしながら凍結法では生体内酵素を不活性化させるまでにかなりの時間を必要とし、この間に測定物質が変化するおそれがあります。

この解決方法としてマイクロウェーブの瞬時照射により諸酵素を不活性化する方法が広く用いられるようになりました。照射後は凍結法で行なわれる低温処理の必要もなく、室温にて処理ができ、安定した測定値が得られます。特に部位別の測定を行なう場合には大変有用です。

●アセチルコリン ●サイクリックAMP ●サイクリックGMP ●GABA ●DOPA ●5-HTP ●セロトニン ●カテコールアミンとその代謝産物 ●エンドルフィン ●プロスタグランディン

などの正確な測定の前処理装置として、薬理学・生化学・生理学・内科学など広い分野に御活用いただけます。



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多チャネル高速処理を活かす

Signal BASIC完成

医用データ処理をリードする7Tシリーズの最高級機7T17は、CPUの効率を飛躍的に向上させる新技術マイクロプログラミング方式により、抜群の高速性とフレキシビリティを実現しました。生体データの能率的な多チャネルオンライン処理が行なえます。

- ■入力チャネルは高速(DC～100KHz)4ch、低速(DC～8KHz)16ch装備
- ■Signal BASICで多チャネルオンライン処理のプログラムを作成可能
- ■豊富なアプリケーションプログラム
- ■ワイドな12インチCRTはチラツキのないラスタスキャン方式
- ■ゆとりある実装メモリ容量512KByte
- ■プログラムやデータのファイルに便利なフロッピィディスク(8インチ)を内蔵
- ■画面は総てサーマルプリンタ(標準付属)でハードコピー

7T17

シグナルプロセッサ

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☎03(209)0811代表

aika

ラット・マウスからウサギ・ネコにまで適用できる小動物実験用ベンチレータ

EVM-50Aは、従来のEVM-50をもとに新たに設計された、主にラット・マウスを対象としたタイムサイクル／フロープリセット方式の人工呼吸器です。

特長

- ●電子コントローラで駆動される吸気／呼気電磁弁によってコンスタントフローを制御するため、長時間にわたって安定した作動を維持できます。
- ●バルブボックスをtype 2（オプション）に交換することによって、ウサギ・ネコにも使用できます。
- ●呼吸回数と吸気時間の設定値がデジタルで表示されるため、正確な設定が行えます。
- ●EIPを0～70%の範囲で設定することができます。
- ●麻酔器との組合せによって吸入麻酔を実施できます。
- ●呼吸回数、吸気時間、EIPの設定ミスまたは電源投入時など、全呼吸時間の80%が過ぎても呼気弁が開かない場合に点灯する警報ランプを備えています。

仕様

サイズ：コントローラ：126mmW×340mmD×244.2mmH
バルブボックス：82.2mmW×82.2mmD×132mmH

重　量：コントローラ：5.6kg
バルブボックス：1.3kg（type 1）

小動物実験用人工呼吸器

アイカ・ベンチレータEVM-50A

株式会社アイカ
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D.S.K MICROSLICER DTK-2000

(特許出願中)

組織・細胞化学用の切片として、凍結または未凍結切片が用いられますが、凍結・融解の過程は細胞の微細形態を破壊するため、できれば未凍結切片を使用すべきであることはよく知られています。しかし、従来の未凍結切片作製用ミクロトームには、組織の破壊が大きく、切片の厚さが一定しない機種や、切片作製に極端に時間がかかり大きな切片や薄い切片が切りにくい機種が多く、また輸入品で高価である等種々難点がありました。弊社ではこれらの欠点をすべて克服した、画期的な未凍結切片作製用ミクロトームとして「マイクロスライサー」を開発しました。

〈応用〉
- 組織化学・細胞化学
  特に電顕レベルの酵素組織化学
- 免疫化学
- 生理学
- 神経化学
- 病理組織検体
- その他一般組織学・細胞学
- 植物組織学

■特長

- 切片作製速度が従来の数倍早くなり、労力が著しく軽減されました。
- 従来よりも、より薄く、より大きな切片が作成できます。
- 柔かい組織、バラバラになりやすかった不均一な組織も切りやすくなりました。
- 輸入品よりも優れた性能と半額以下の価格を実現しました。

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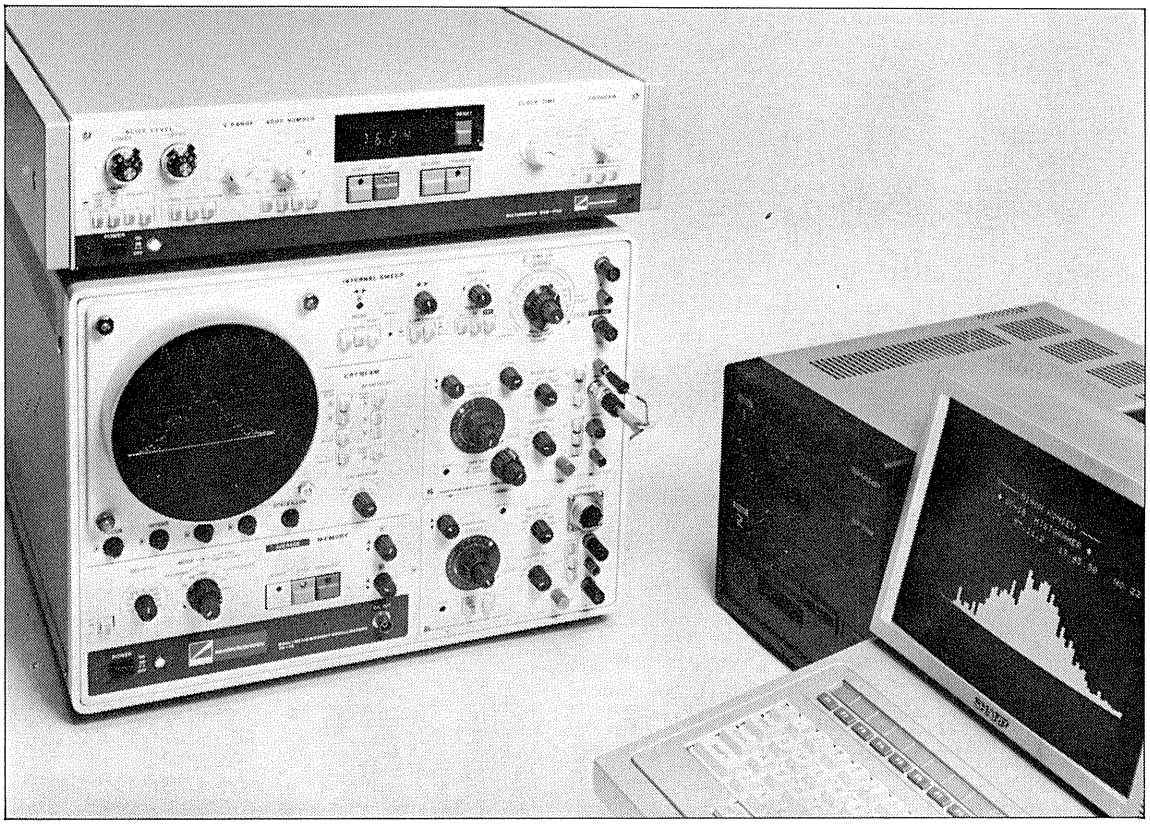
アベレージ・ヒスト…
拡張性を秘めたVC-10。



〔2-4現象 メモリオシロスコープ VC-10〕

■VC－9の使い易すさをそのままにメモリオシロにグレードアップしたVC－10 ■2チャネルメモリを内蔵、アベレージャヒストグラムユニットの追加が可能 ■それに加え、これらの出力をパソコンへ接続するためのインターフェイスを内蔵等、大きな拡張性を秘めています。

〈オプション〉

■アベレージャ DAT-1100

チャネル：2チャネル
A/D変換：8ビット
メ モ リ：16ビット×1024ワード×2ch
出　　力：CRT用，直記式レコーダ用，XYレコーダ用，
　　パソコン用（インターフェイス内蔵）

■ヒストグラムユニット DAB-1100

チャネル：1チャネル
処理プログラム：INTERVAL
　　DWELL TIME
　　LATENCY
　　PULSE COUNT
　　PULSE HEIGHT
　　（SEQUENTIAL及び NON SEQUENTIAL）
スライサ：ウインド型スライサ内蔵
出　　力：アベレージャに同じ

エレクトロニクスで病魔に挑戦する

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